CHEMICAL CONSTITUENTS OF *WALSURA PINNATA* HASSK

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FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

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MAHFUZAH YUSOFF

DISSERTATION SUBMITTED IN FULILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR
2012

UNIVERSITI MALAYA

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ABSTRACT

The chromatographic separation of the *n*-hexane extract from the stem bark of *Walsura pinnata* Hassk (Meliaceae) which was collected at Kuala Lipis, Pahang, led to the isolation of nine chemical constituents. Their structures were characterized to be ledol (**115**), oplopanone (**116**), betulonic acid (**117**), oleanonic acid (**118**), 3-oxoolean-11-en-13β(28)-olide (**119**), 12α-hydroxy-3-oxooleanano-28,13-lactone (**120**), 3-oxoursolic acid (**121**), 3-ketours-11-en-13β(28)-olide (**122**), 3β-hydroxy-5-glutinen-28-oic acid (**123**). The structural elucidation of these compounds was determined by spectroscopic data interpretation. Betulonic acid (**117**) and 3β-hydroxy-5-glutinen-28-oic acid (**123**) were found to be cytotoxic against a series of human cancer cell lines with an IC_{50} = 19 μM, 18 μM on HepG2; 12 μM, 25 μM on MCF-7 and 13 μM, 21 μM on HSC-2 respectively.

ABSTRAK

Pemisahan kromatografi ekstrak heksana daripada kulit pokok *Walsura pinnata* Hassk (Meliaceae) yang diperoleh dari, Kuala Lipis, Pahang telah menghasilkan sembilan sebatian kimia. Strukturnya telah dikenalpasti sebagai ledol (**115**), oplopanon (**116**), asid betulonik (**117**), asid oleanonik (**118**), 3-oksoolean-11-en-13β (28)-olid (**119**), 12α-hidroksi-3-oksooleanan-28,13-lakton (**120**), asid 3-oksoursolik (**121**), 3-ketours-11-en-13β (28)-olid (**122**), asid 3β-hidroksi-5-glutinen-28-oik (**123**). Penentuan struktur sebatian organik ini adalah melalui kaedah spektroskopi. Asid betulonik (**117**) dan asid 3β-hidroksi-5-glutinen-28-oik (**123**) didapati mempunyai aktiviti sitotoksik terhadap beberapa sel kanser manusia dengan nilai kepekatan 50 peratus perencatan (IC_{50}) masing - masing adalah = 19 μM, 18 μM pada sel HepG2; 12 μM, 25 μM ke atas sel MCF-7 dan 13 μM, 21 μM pada sel HSC-2.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisors, Profesor Dr. Khalijah Awang and Assoc. Prof. Dr. Khalit, on their supervision, advices, patience and guidance throughout the course of this study. My appreciation also extends to Associate Professor Dr. Hasima Nagoor for her help on biological activity.

Thanks to all the staff from Chemistry Department, University Malaya, especially Pak Din, Mr. Teo, Abg. Rafly, Kak Nor, Kak Fiona, Kak Sue, and Mr. Siew. Without their help, this research work could not be completed.

I wish to express my gratitude to my family, especially my beloved husband Mr. Luqman Hakim, my princess Nur Khairunnisa Alisha, my parent, Mr. Yusoff and Mrs. Rodiyah and my brothers and sisters.

Last but not least, I would like to forward my thanks to all Phytolab and C-10 lab members; Abg. Chong, Abg. Fazli, Abg. Nazif, Kak Vicky, Abg. Lan, Kak Tini, Kak Dewi, Kak Cewek, Kak Nita, Kak Yasodha, Chan, Aimi, Lailey, Faizah, Nana, Julia, Rosalind, Ximen, Tiong, Remy, Tien, Omer, Farhana, Azmi, Linda, Nadia, Azeana, Ayu, Shelly and Nurul.

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ABBREVIATIONS

α	Alpha
β	Beta
γ	Gamma
λ	Lambda (maximum wavelength)
μ	Micro
$CDCL_3$	Deuterated chloroform
cm^{-1}	Per centimetre
δ	Chemical shift
DEPT	Distortion less Enhancement by Polarisation Transfer
HMQC	Heteronuclear multiple quantum coherence
HMBC	Heteronuclear multiple bond coherence
s	Singlet
d	Doublet
dd	Doublet of doublets
t	Triplet
m	Multiplet
Hz	Hertz
J	Coupling constant
NMR	Nuclear magnetic resonance

^{1}H	Proton NMR
^{13}C	Carbon-13NMR
m/z	Mass per charge
IR	Infrared
UV	Ultraviolet
LCMS	Liquid chromatography mass spectrometry
ESI	Electrospray ionization
EIMS	Electron ionization mass spectrometry
GCMS	Gas chromatography mass spectrometry
MS	Mass spectrometry
HPLC	High performance liquid chromatography
L	Litre
ml	millilitre
ppm	Part per million
MHz	Megahertz
g	Gram
mg	Milligram
µg	Microgram
µM	Micro molar
µL	Micro litre

H-	Proton
CH_3/ Me	Methyl
C-	Carbon
TLC	Thin layer chromatography
CC	Column chromatography
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
CaSki	Cervical
MCF-7	Carcinoma cell lines breast
HSC2	Nasopharyngeal
HSC4 (Cox2)	Nasopharyngeal
HepG2	Liver
HMEC	Normal cell line
IC_{50}	Half maximal inhibitory concentration

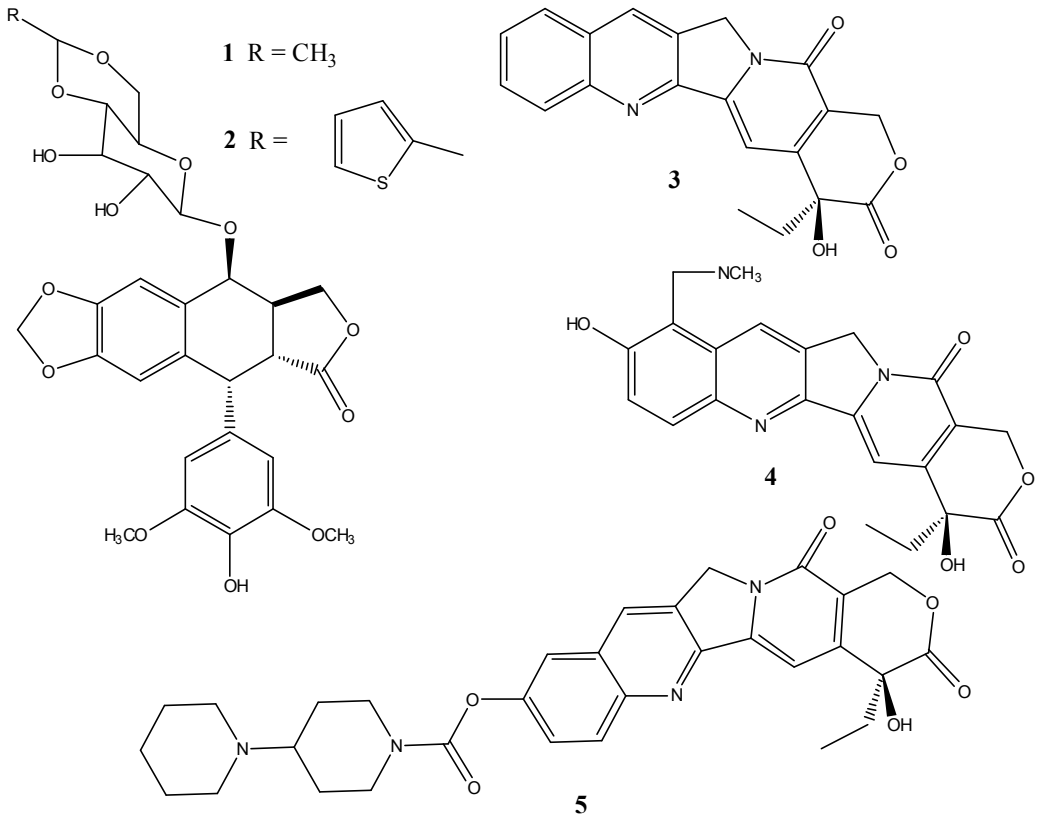
CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

World Health Organisation (WHO) has defined medicinal plants as plants that contain some organic compounds or properties which produce definite pharmacological action on the human body that can be used for therapeutic purposes (WHO 2008) and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[1,2]. Medicinal plants have great and important roles as mediator of ecological interactions; but in most cases, the substances appear to serve no useful purpose in the microorganisms producing it[2,3].

Natural products have served as the source and inspiration for a large fraction of the current pharmaceuticals. Although estimates is very dependent on the definition of what is considered a natural product derived drug, it is safe to say that between 25% and 50% of currently marketed drugs owe their origin to natural products[4]. In addition to their direct use as drugs, many natural products have served as lead compounds for medicinal chemistry. In the case of anticancer and anti-infective agents the proportion is even higher, and the estimate is that almost two-thirds of such agents are derived from natural products[5]. In anticancer area the lead compound podophyllotoxin led to the clinical drugs etoposide **1** and teniposide **2**, and the lead compound camptothecin **3** spawned the drugs topotecan **4** and irinotecan **5** [6].



The use of plants as the cure of many ailments by the local people have been well documented as early as 1935[7]. Medicinal plants were also available at that time in the markets or sidewalks, mostly in crude form, sold by traditional medicine practitioners. It was not until the 1950s that any attempt was made to carry out scientific investigation on these medicinal plants although awareness on the usefulness of such plants has been established for quite some time. Research at that time was simple and straightforward but effective which involved surveys of plant species available in a certain designated area, their ethno botanical use, and laboratory testing for certain classes of compounds which were already known to be effective drugs. So it was not surprising then that most of the phytochemical surveys carried out concentrated on the testing of alkaloids in these plants since alkaloids were already proven to be effective as drugs.

A drug takes several years and millions of dollars to be developed, hence making the process very capital-intensive, the risks are also high and the success rate is not very good. Despite all these, natural products drug discovery programmes are still in existence all over the world, mainly because of the high chemical diversity from natural

products as compared to synthetics, and the potential of these natural products is largely known. Moreover, the large number of terrestrial and marine species yet uninvestigated, and the back to nature trend. In addition, the modern technology and the advancements in this field have made natural products drug discovery programmes attractive. Thus high throughput screens and sensitive instrumentation for example (LCMS, X-ray crystallography, HPLC etc) for structure elucidation has reduced significantly the amount of time (and also the amount of sample) required for the first stage of the investigation.

In Malaysia, 2000 species from 14500 flowering plants have been reported to contain medicinal properties and many have been scientifically proven[8]. Malaysia is wealthy in her plant diversity and potential lead compounds and therapeutics are still waiting to be explored. The huge diversity of the Malaysian flora means that we can expect well diversed chemical structures from their secondary metabolites, and chemical diversity is one of the plus factors that makes natural products excellent candidates for any screening programme. Plant derived compounds offer potential source of new antimicrobial, anticancer and anti-HIV compounds among other pharmaceuticals[9].

1.2 MELIACEAE

The Meliaceae is a medium-sized family of woody plants growing in the tropics and subtropics, comprising 51 genera and about 575 species of trees and rare shrubs. Most members of the family have large leaves, with the leaflets arranged in the form of a feather, and branched flower clusters. The fruit is fleshy and coloured or a leathery capsule. This family is extremely useful to man for the high quality timbers and ease with which some species can be grown in plantation[10]. Generic monograph of the Meliaceae, divided Meliaceae into four subfamilies as shown in Figure 1.2a and 1.2b.

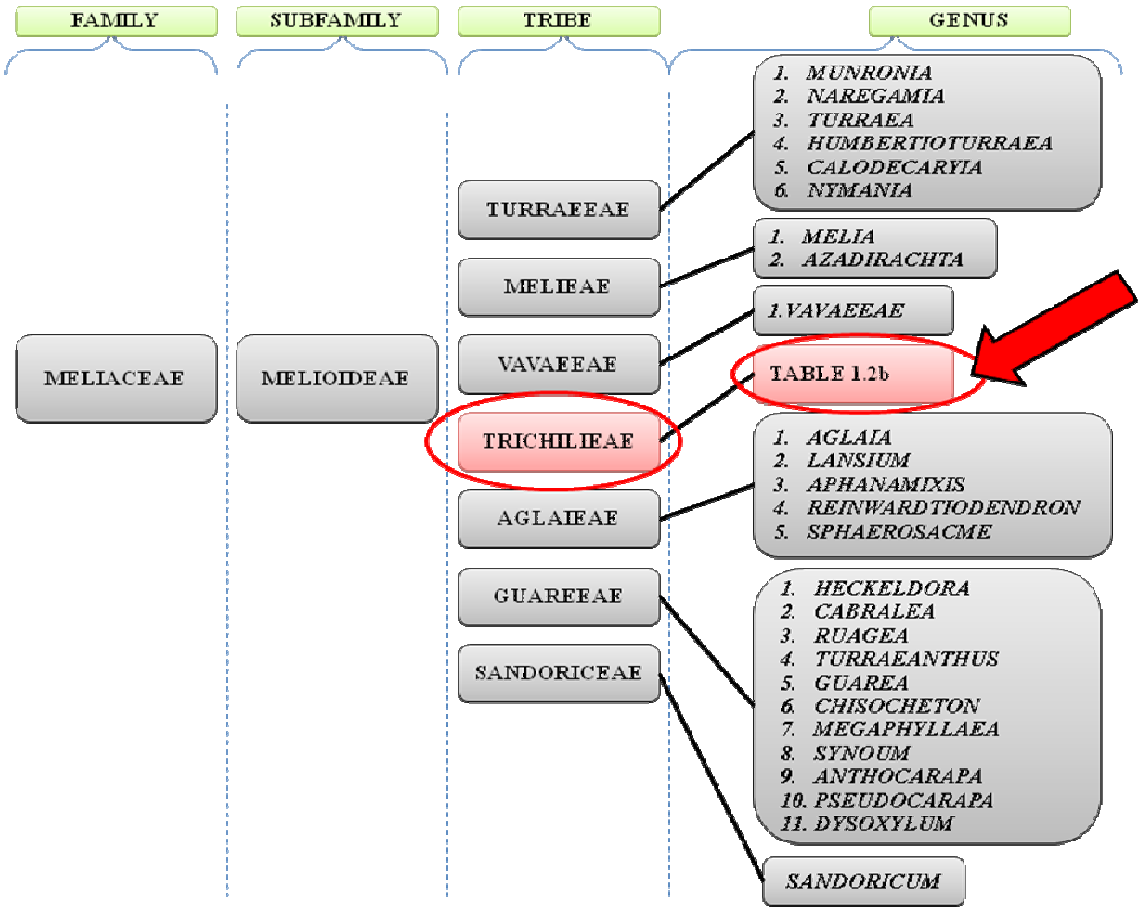


Figure 1.2a : Classification of Meliaceae Family

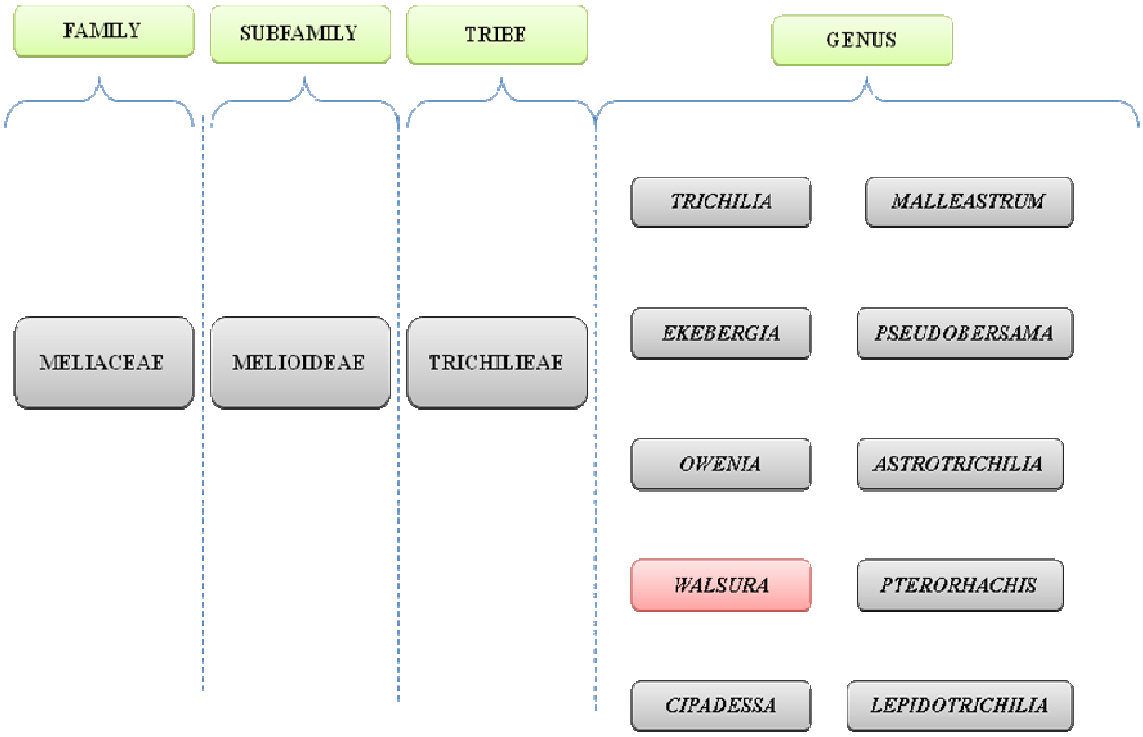


Figure 1.2b : Genus from Trichilieae tribe.

1.3 THE CHEMISTRY OF MELIACEAE

The Meliaceae produce wide range of compounds, flavonoids, chromones, coumarins, benzofurans, mono-, sesqui-, di- and triterpenoids, but are best known for the production of limonoids which are tetranortriterpenoids with β-substitued furanyl ring at C17α. Alkaloids are rarely isolated from the Meliaceae[11].

The bioactivity of compounds from Meliaceae has been the subject of past reviews[12]. Extracts from *Ekebergia capensis* are important in Zulu traditional medicine in South Africa, being used to induce or facilitate labour in pregnant women and have been shown to have uterotonic activity[13]. Bark from both *Trichilia dregeana* and *Trichilia emetic* is used to procure abortions and is used as a fish poison in Zimbabwe[14]. Preparation from Neem tree (*Azadirachta indica*) are used to treat a wide range of conditions and the plant yields the insect anti-feedant compound azadirachtin[12]. The neem, or nim tree is also called the margose tree, grown throughout the Old World tropics, notably in India and Southeast Asia, as a source of timber and medicinal oils and resin.

1.4 GENUS *WALSURA*

The genus *Walsura* comprising about 40 species and varieties is mainly distributed in Southeast Asia and China[15]. Previous studies on this species have resulted in the isolation of various compounds such as, triterpenoids and tetranortriterpenoids with a variety of biological activities such as antibacterial, antigardial, antioxidative, antimalarial, and antifeedant[15-17].

1.5 OBJECTIVES OF STUDY

The objectives of this study are as follows:

I. To isolate the chemical constituents from *Walsura pinnata* Hassk.

II. To perform fractionation and isolation of chemical constituents from the plants samples using chromatographic techniques.

III. To identify each isolated constituents by means of spectroscopic method such as NMR, IR, and MS.

IV. To perform cytotoxicity assay on selected pure compounds using various human tumour cell lines.

CHAPTER 2

GENERAL CHEMICAL ASPECTS

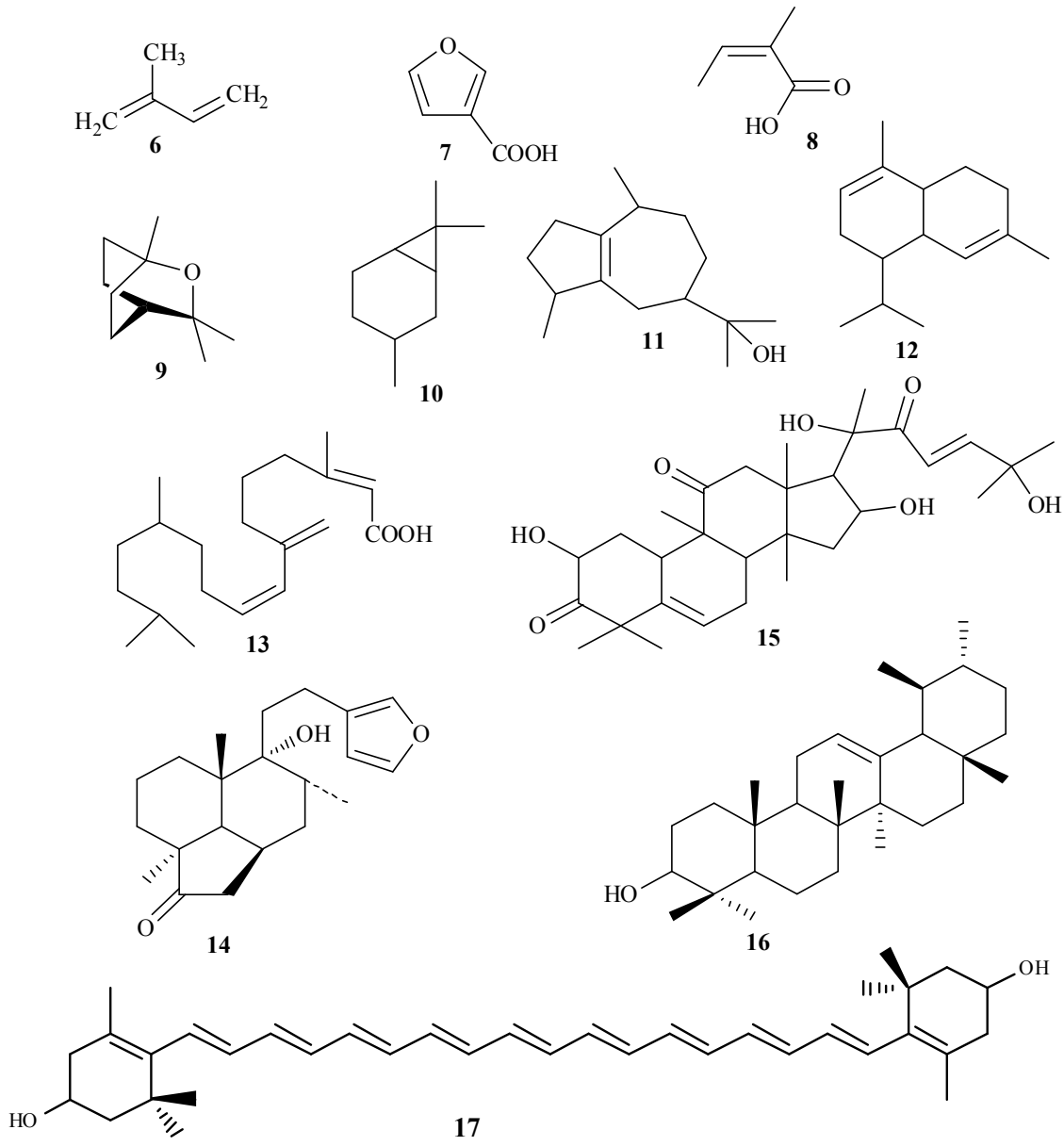
The following sections will discuss briefly some of terpenoids. The genus *Walsura* are known to produce a variety of chemical compounds such as triterpenoids and tetranortriterpenoids.

2.1 TERPENOIDS

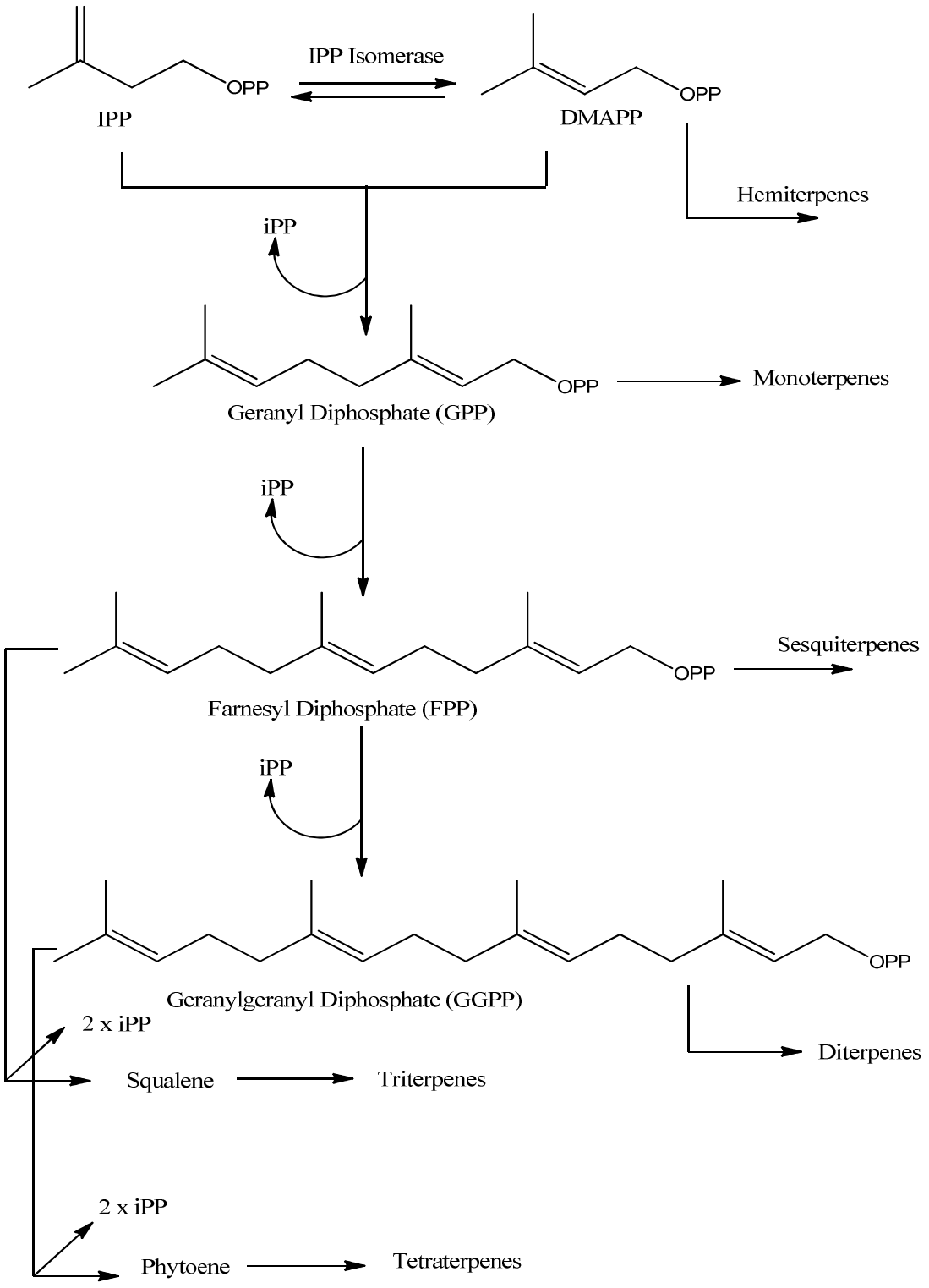
Terpenoids are structurally diverse group of natural products. More than 25000 representatives with a variety of biological functions have been reported in the plant kingdom such as carotenoids serve as light-harvesting and light-protecting pigments, sterols play an important role as modulators of membrane properties, the phytol side chain of chlorophyll (the most abundant organic pigment) is of terpenoid origin, and a wide variety of plant terpenoids function as insect attractants or repellants[25]. Various terpenoids have attracted commercial interest as pharmaceuticals or nutraceuticals. Terpenoids are a unique group of hydrocarbon based natural products whose structure may be derived from isoprene **6**. So, terpenes are classified as follows by starting from 5 carbon units as shown in Table 2.1

Table 2.1 : Classification of Terpenes

Bil	Number of Carbon	Class	Example
1	5	Hemiterpenes	β – Furoic acid **7**, Angelic acid **8**
2	10	Monoterpenes	Cineole **9**, Carane **10**
3	15	Sesquiterpenes	Guaiol **11**, α – Cadinene **12**
4	20	Diterpenes	Phytol **13**, Marrubin **14**
5	30	Triterpenes	Cucurbitacin D **15**, α–Amyrin **16**
6	40	Tetraterpenes	Lutein **17**



Like all natural products, within this simple classification lies an enormous amount of structural diversity, which leads to a wide variety of terpene like compounds. The simplest examples of the terpenes are technically hydrocarbons. The terpenes are of a similar biogenetic origin, in which repetitive addition of isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) combine produces geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl diphosphate (GGPP). Two molecules of FPP on addition give triterpenes whereas addition of two molecules of GGPP gives tetraterpenes (Scheme 2.1). These various combinations and oxidations give rise to a large variety of terpenes.



Scheme 2.1 : Isomerization of IPP to DMAPP and Repetitive IPP addition to give Higher Terpenoids[26].

2.2 SESQUITERPENOIDS

The C_{15} sesquiterpenes was derived from three isoprene units and exist in a wide variety of forms, including linear, bicyclic and tricyclic frameworks. Most of the sesquiterpenes are considered to be essential oils because they belong to the steam distillation fraction often containing the characteristic odoriferous components of the plant. Some common sesquiterpenes are shown in Figure 2.2 such as bisabolenes, cadinenes, humulenes, santonins and etc.

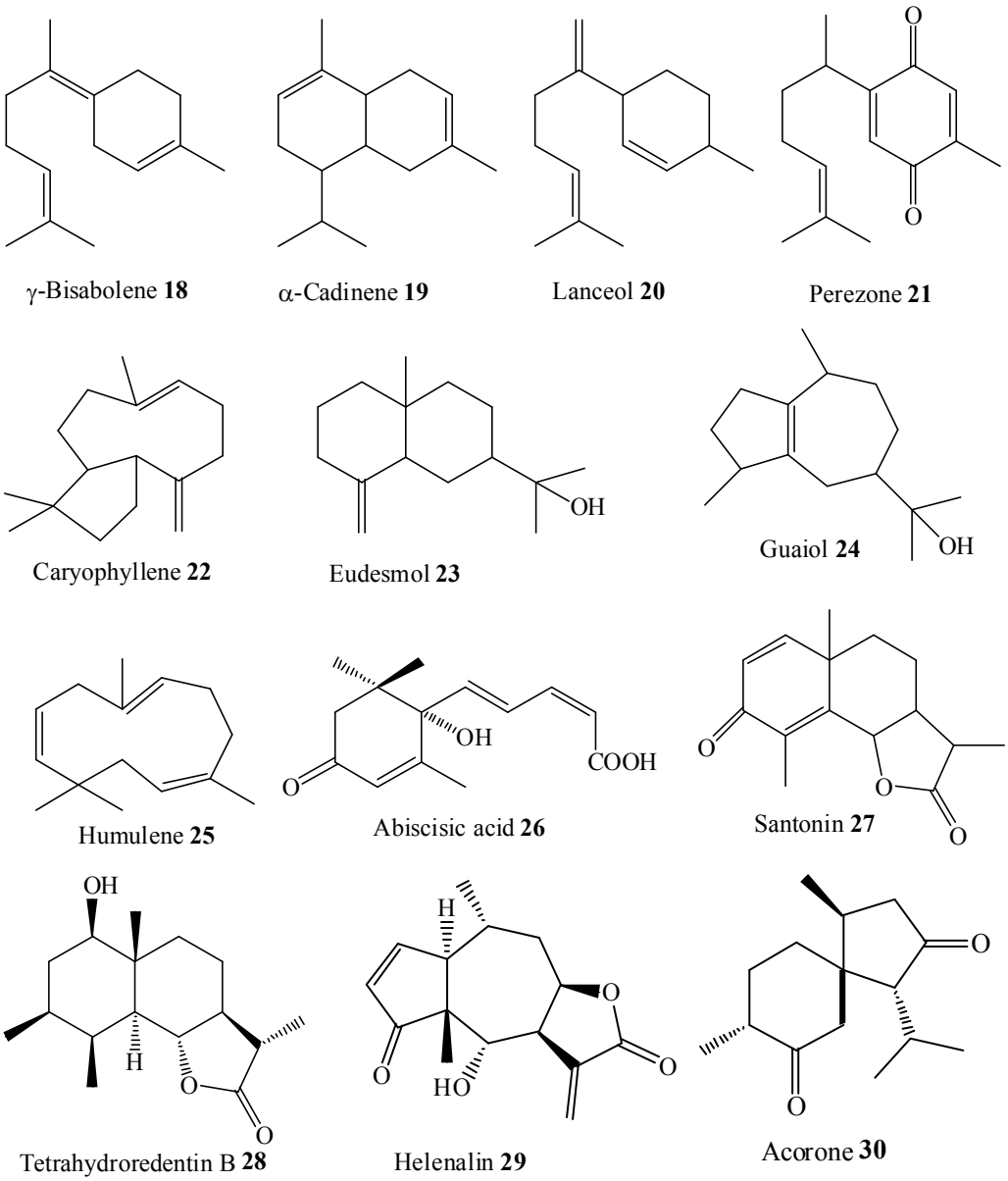
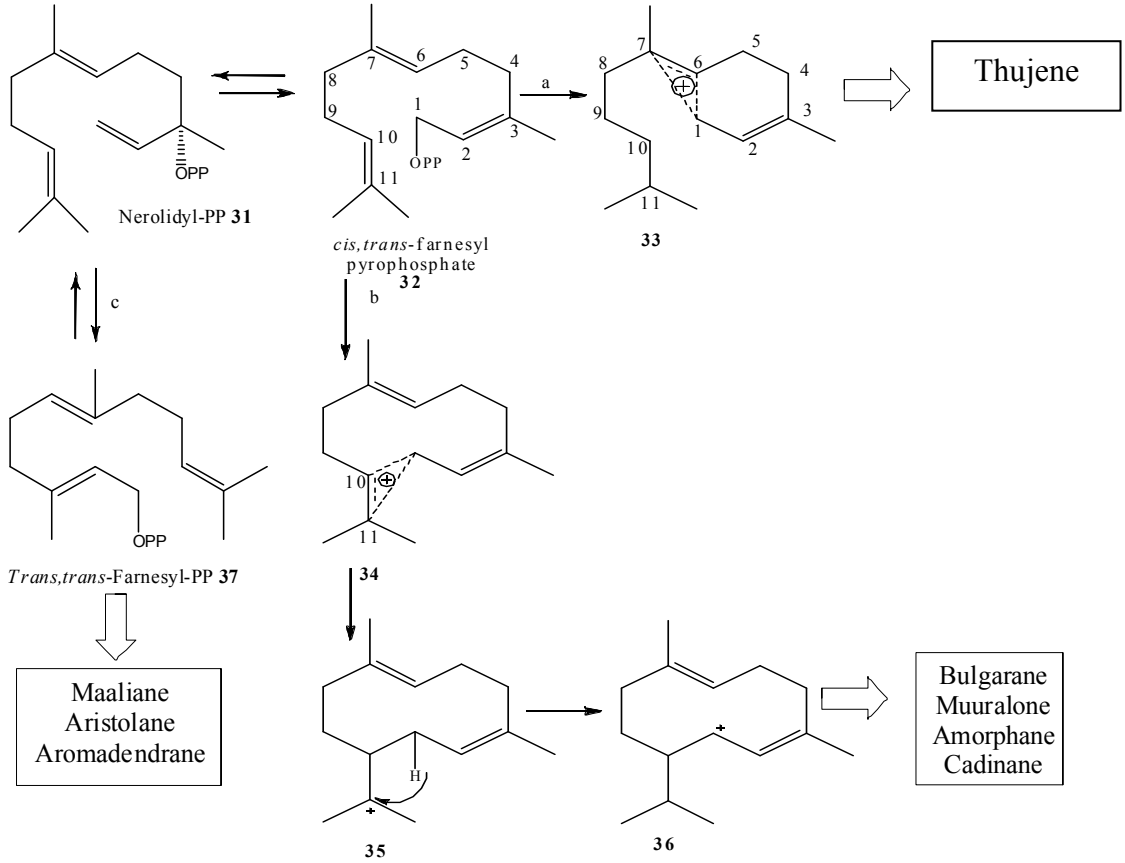


Figure 2.2: Some important Sesquiterpenoids.

The santonin **27** is an antihelminthic that is isolated from wormwood (*Artemisia maritima*). Caryophyllene **22**, first synthesized in 1964, is one of the principal components of oil of cloves. Helenalin **29** is one of numerous pseudoguaianolide sesquiterpene lactones isolated from arnica oil (*Arnica Montana*). Acorone **30** is a sesquiterpene diketone present in the essential oil of sweet flag (*Acarus calamus*). The sesquiterpene α-cadinene **19** is one of the more than 70 isolated compounds from the essential oil of juniper berries. Finally, tetrahydroridentin B **28** is one of the bitter eudesmolide unique to the common dandelion (*Taraxacum officinale*).

The biosynthesis of sesquiterpenes can be discussed as follows: a) sesquiterpenoids from *cis*,*trans*-farnesyl pyrophosphate with initial closure at 6,7-double bond **33**, b) sesquiterpenoids from *cis*,*trans*-farnesyl pyrophosphate with initial closure at 10,11-double bond of **34** and c) sesquiterpenoids from *trans*,*trans*-farnesyl pyrophosphate **37**. The typical sesquiterpene skeletons and their biogenetic relationship is shown in Scheme 2.2

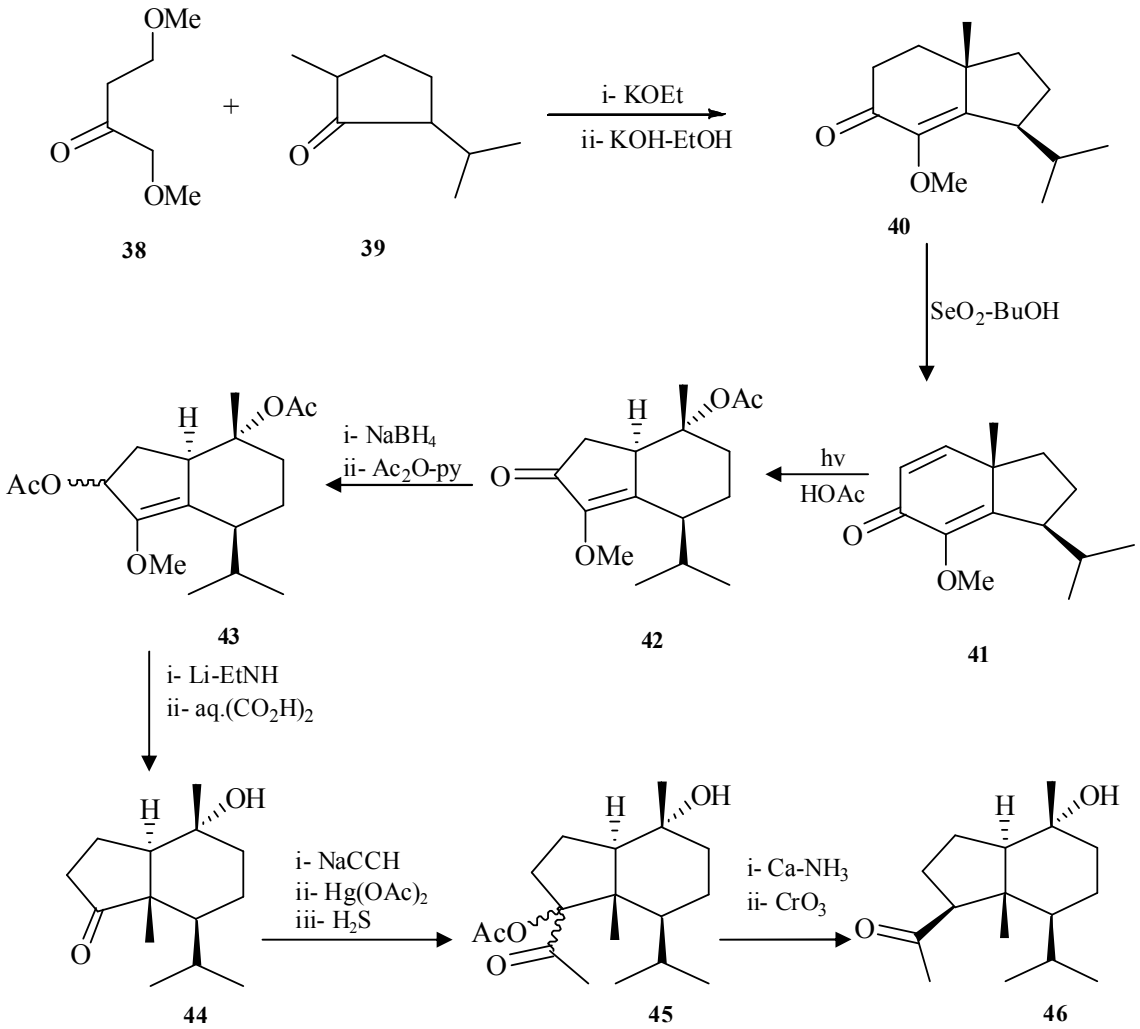


Scheme 2.2: Formation of some sesquiterpenoid skeleton through carbocations/ions pair intermediates[26].

The following sub-chapter shall discuss briefly about cadinane and aromadendrane type of compounds.

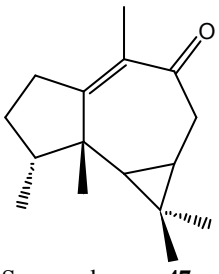
2.2.1 CADINANE GROUP

The biosynthesis of the cadinane skeleton (Scheme 2.2.1) which can be derived from carbonium ions **36**, this in turn arise from *cis, trans*-farnesyl pyrophosphate **32** via the non-classical ion **34**. This skeleton can also be established by synthesis (Scheme 2.2.1) in racemic form using a key photochemical step that lead to the formation of ring contracted cadinane sesquiterpenoid, example oplopanone **46** from *Oplopanax japonicus*[27].



Scheme 2.2.1: Synthesis of cadinane group[28].

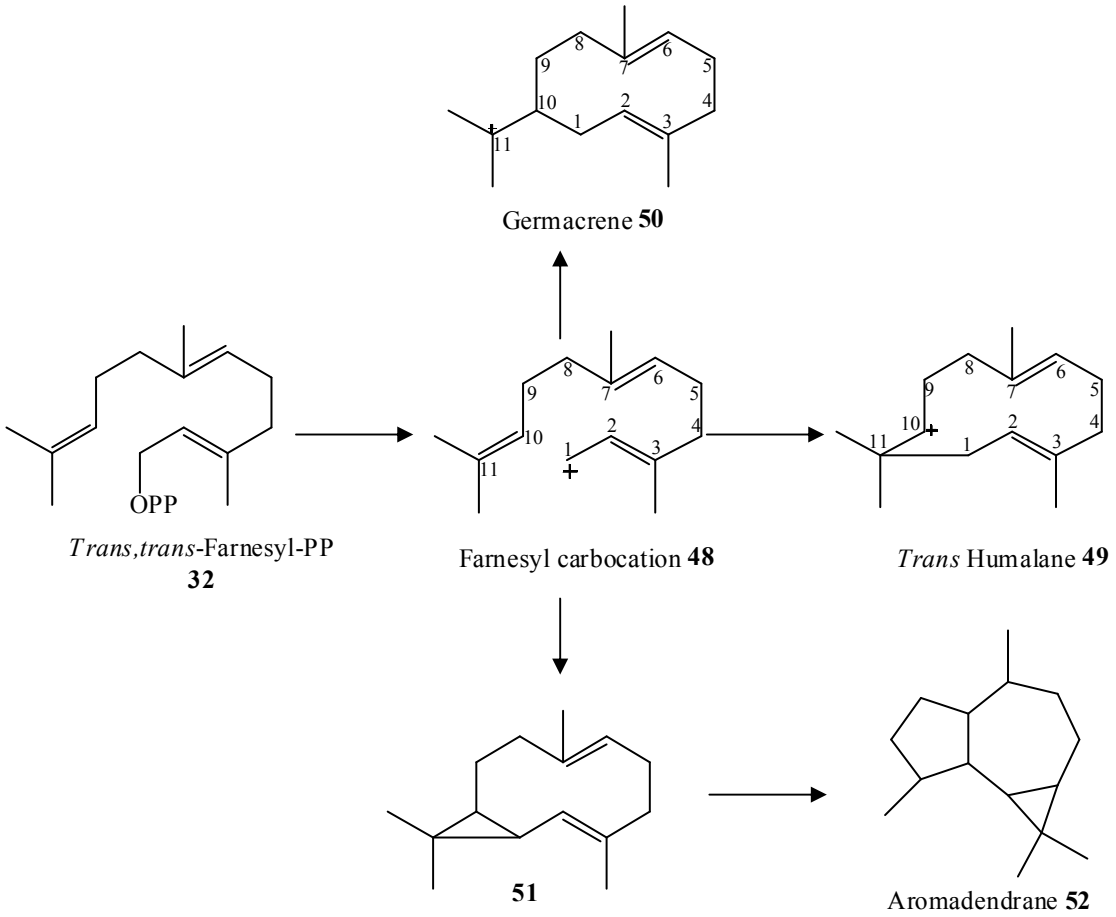
2.2.2 AROMADENDRANE GROUP



Squamulosone **47**

The farnesyl carbocation **48** (Scheme 2.2.2) in which both double bonds are in the *trans* configuration, can add to the 10,11-double bond, either at the 11-carbon to give *trans* humalane **49** or the 10-carbon to give the germacrane **50** skeleton and third

possibility is to insert into the 10,11-double bond to give a cyclopropane ring. The ring strain in the resultant material can be relieved by *trans* annular cyclization **51** to give the aromadendrane **52** skeleton, example squamulosone **47** from *Phebalium squamulosum*[29].



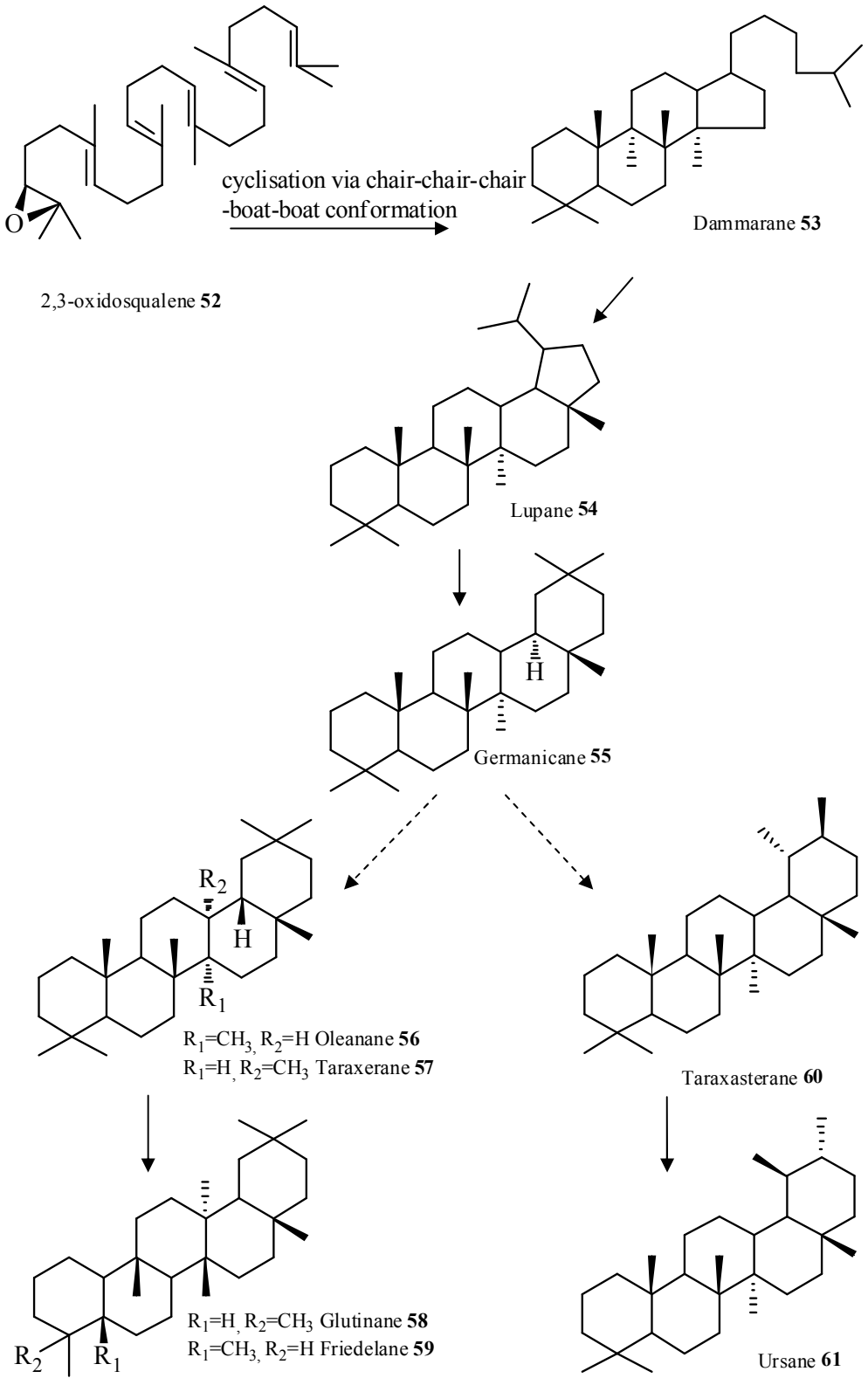
Scheme 2.2.2 : Sesquiterpenoids from *trans,trans*-farnesyl[26].

2.3 TRITERPENOIDS

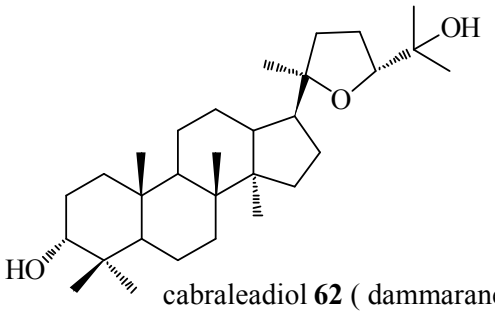
Triterpenoids form the largest and most widely distributed terpenoids class. For the most part they have four or five rings, with double bonds, hydroxyl, carboxyl, ketonic groups, acetoxyl group, oxide ring, lactone and acid groups. They occur mainly in plants, often as esters or glycosides, but the class also includes most of the few known terpenoid substances of animal origin.

Most triterpenoids fall into one of two major classes, which is tetracyclic series of non-isoprenoid compounds structurally related to the steroids, (trimethylsteroids),

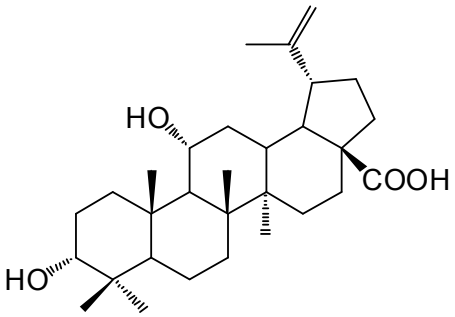
some of which have 31 carbon atoms and a very large pentacyclic class comprising a number of skeletal types (Scheme 2.3) and some of their skeleton are depicted in Figure 2.3. The second class is called 'nortriterpenoids', which possess less than 30 carbon atoms, but appear to be biogenetically related to the C_{30} series.



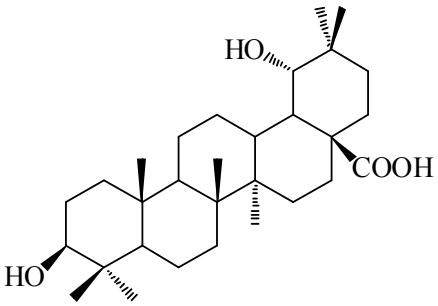
Scheme 2.3: Schematic Correlation Of Main Triterpenoids Skeleton[26]



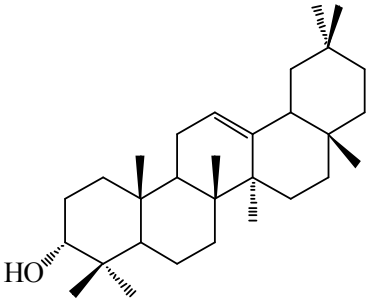
cabraleadiol **62** (dammarane)[19]



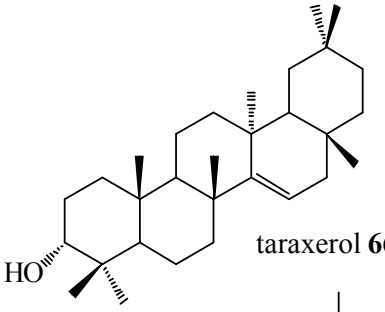
acantrifoside A **63** (lupane)[23]



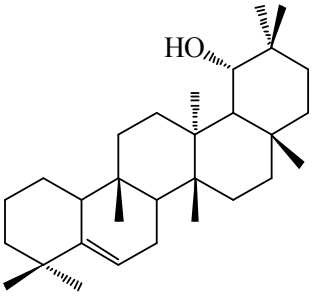
Machaeroceric acid **64** (germanicane)[18]



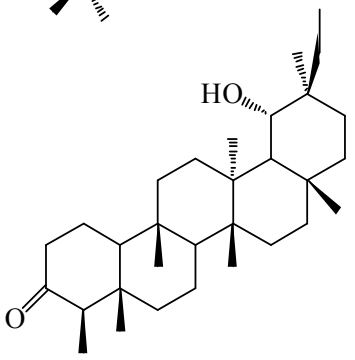
3-*epi*-β-amyrin **65** (oleanane)[22]



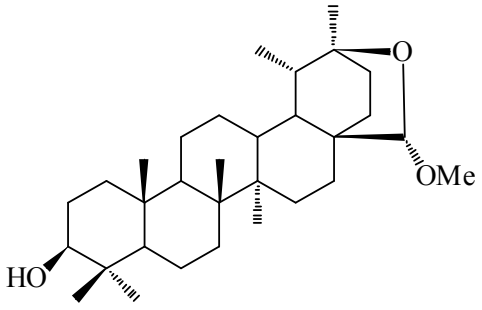
taraxerol **66** (taraxerane)[22]



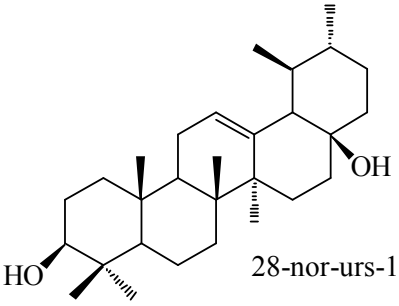
19α-glutin-5-en-19-ol **67** (glutinane)[21]



3-oxofriedelane **68** (friedelane)[24]



20β,28-epoxy-28α-methoxytaraxasterane-3β-ol **69** (taraxasterane)[20]



28-nor-urs-12-ene-3β,17β-diol **70** (ursane)[20]

Figure 2.3: Some examples of triterpenoids skeleton.

The following paragraphs shall discuss briefly about the subgroups of related triterpene.

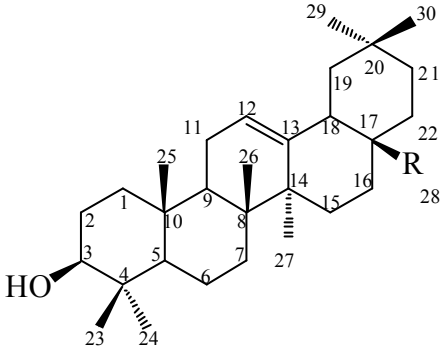
2.4 PENTACYCLIC TRITERPENOIDS

Pentacyclic triterpenes constitute an important group of natural compounds. They are obtained from plants and especially from bark of trees such as plane, cork and birch but also in liquorice roots where they are particularly abundant. Pentacyclic triterpenoids based on the oleanane, lupane and related skeletons have provided some of the most useful markers for inputs of organic matter from terrestrial plants to marine sediments. For example, β-amyrin and taraxerol and their degradation products were used as tracer of organic matter from mangroves in sediments from Florida Bay[30].

Its also contributes to the development of modern therapeutics drugs. Their biological properties are considerable[31]. For example, betulinic acid and ursolic acid have been shown to exhibit significant anticarcinogenic and anti-HIV activity[32,33], lupeol is a competitive inhibitor of both trypsin and chymotrypsin[34] and the antiphlogistic activity of betulin was confirmed in various experimental models[35]. α-amyrin and β-amyrin have been patented for use in cosmetic industries as hair and skin protecting agent[36]. More than 200 triterpenoids containing different carbon skeletons and functional groups have already been reported[37].

The following sub-chapter shall discuss briefly three type of pentacyclic triterpenes ; oleanane, ursane and lupane types.

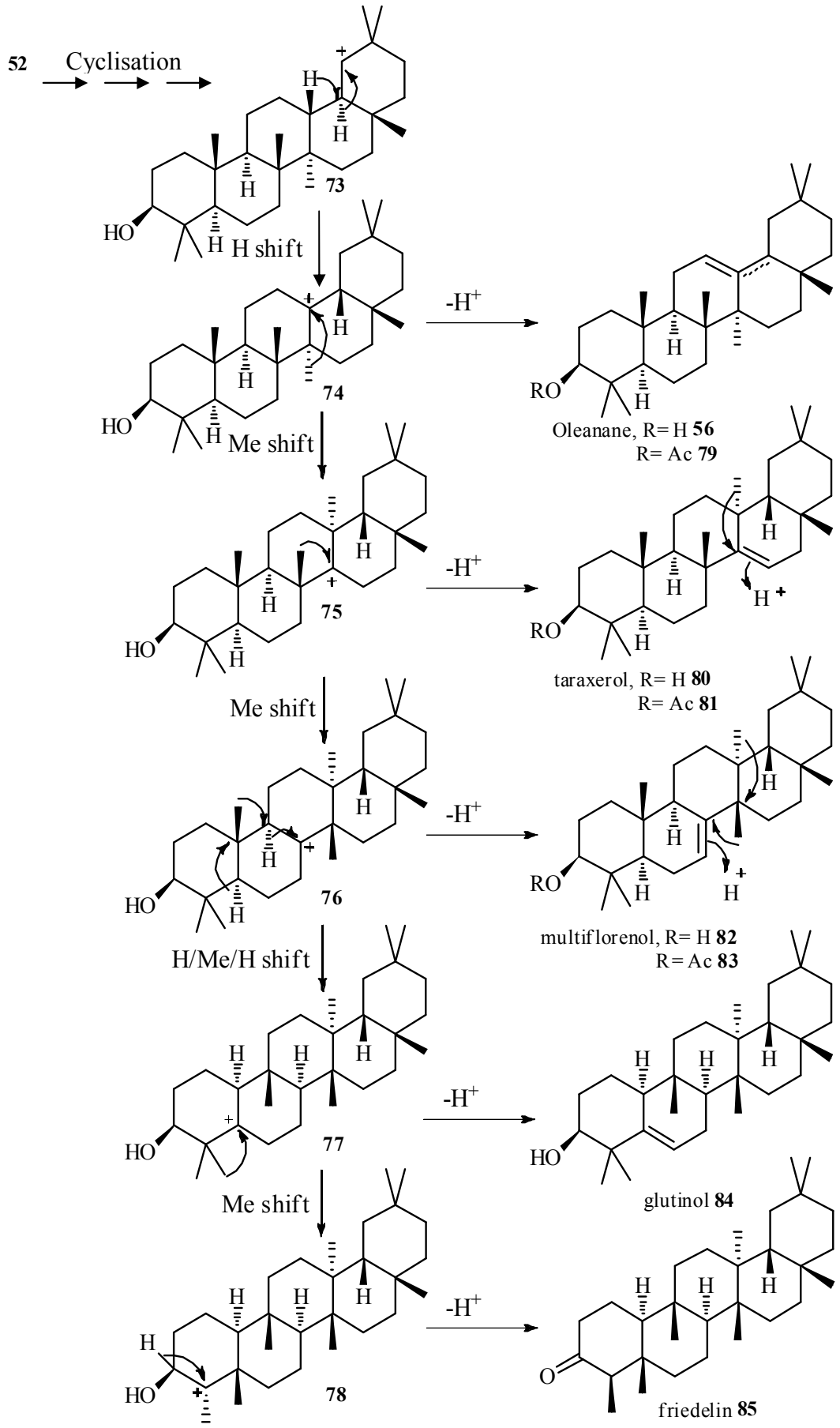
2.4.1 The Oleanane (*β*–Amyrin) Series



R=COOH Oleanolic acid **71**
R=CH_3 *β*-amyrin **72**

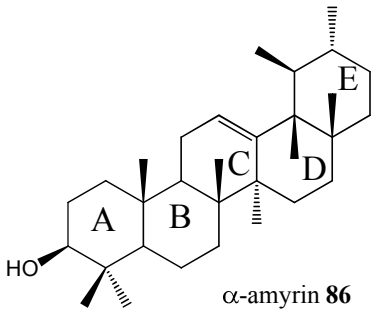
The parent hydrocarbon of this, the largest pentacyclic group, is oleanane which takes its name from oleanolic acid **71**, a constituent (as a glycoside) of olive leaves, mistletoe and cloves. Historically, the series derived from *β*-amyrin **72**, which was originally isolated in the nineteenth century from elemi resin, and is widespread in plants, both free and as esters, for example in grape seeds and alfalfa.

Oleanane derivatives (Scheme 2.4.1) are numerous and widespread in nature, and differ structurally in their degree and position of oxygenation. The C-3 oxygen function is universally present and the C-12, C-13 double bond almost so the former function usually takes the form of a *β*-hydroxyl group, but the α-configuration is sometimes found, and some ketones are known. About 80 percent of natural oleananes are oxygenated at C-28, which is most commonly a carboxyl group, but may be a hydroxymethyl or, occasionally, an aldehyde group. Similar oxygenation is common at C-23, and occasionally found at C-30. Additional hydroxyl groups are commonest at C-2 and at C-16, and occur occasionally in other positions, notably C-21 and C-22[38]. A few lactones and epoxides are also known.



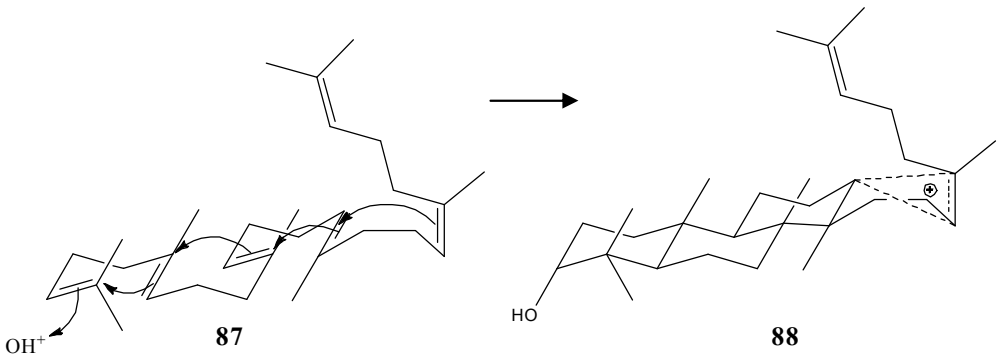
Scheme 2.4.1: Rearrangement of pentacyclic triterpenoids with oleanane skeletons[26].

2.4.2 The Ursane (*α*-Amyrin) Series



α-amyrin **86**

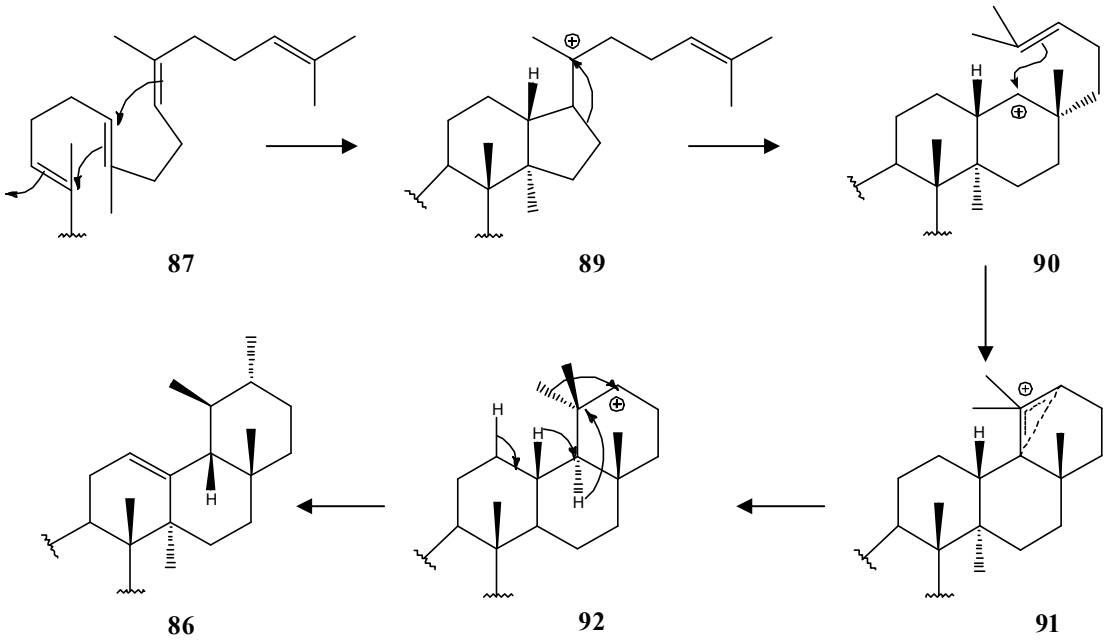
α-Amyrin **86** has many natural sources, and occurs in Manila elemi resin along with *β*-amyrin, from which it differs only in ring E; indeed the two compounds are so similar in properties that they were originally believe to be stereoisomers. The triterpenoid of the ursane series arise, from the chair-chair-chair-boat unfolded conformation **87** of squalene via the non-classical ions **88**. For the sake of clarity and simplicity, the *trans*formations leading to the construction of rings D and E are best illustrated by two dimensional formulae and for the most part, simple carbonium ions shown in Scheme 2.4.2a.



87 **88**

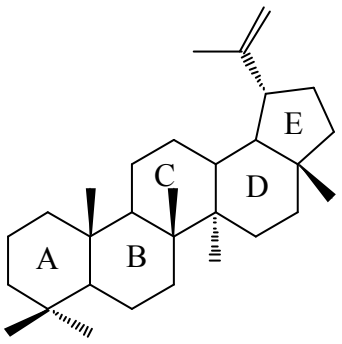
Scheme 2.4.2a: Construction of rings D and E in ursane skeletons[38].

Thus oxidative cyclization of squalene **87** to **89**, followed by ring enlargement, gives **90**, which cyclises to non-classical ion **91**. This giving **92**, which converted by methyl and hydride shifts and deprotonation into α-amyrin **86** shown in Scheme 2.4.2b.

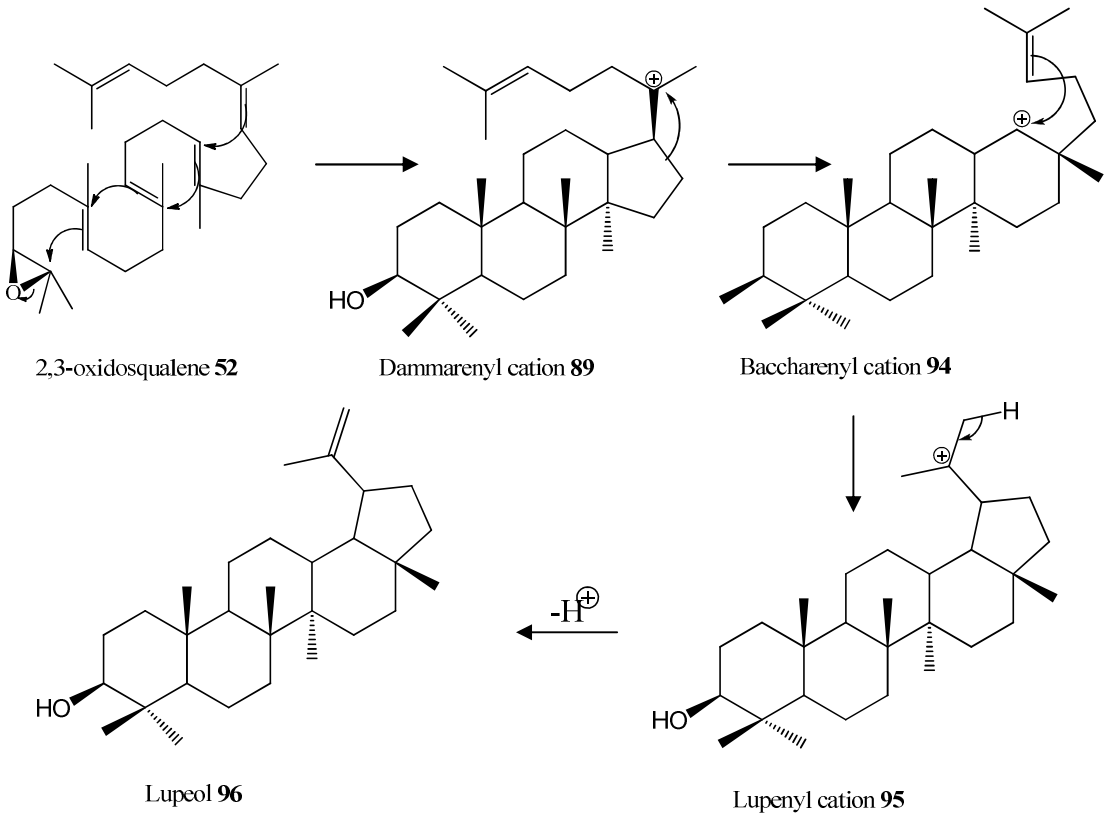


Scheme 2.4.2b: Cyclization of squalene to give ursane skeleton[39].

2.4.3 The Lupane Series



Lupeol **96**, was isolated from lupin seeds, and is very widely distributed in plants. The lupane skeleton arises by the same biosynthetic processes as the ursane and oleanane skeletons, (Scheme 2.3) and lupeol **96** is derivable by proton elimination from the intermediate **95** Scheme 2.4.3.

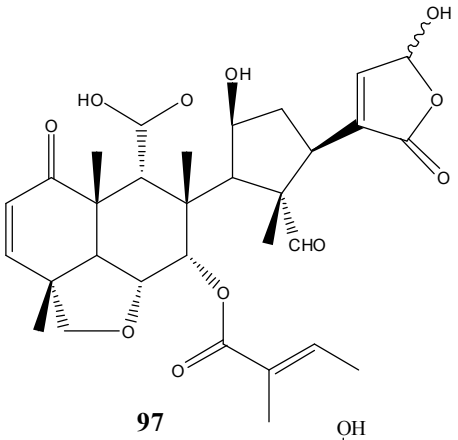


Scheme 2.4.3: Biosynthetic processes of lupane skeleton[39].

2.5 CHEMICAL CONSTITUENTS OF THE GENUS *WALSURA*

Walsura Species	Compounds	Reference
Walsura chrysogyne	Walsogyne A **97**	40
Walsura piscidia	Piscidinol B **98** Piscidinol C **99** Piscidinol D Piscidinol E Piscidinol F **100**	41,42
Walsura robusta	Walsuranoid A **101** Walsuranoid B **102** Walsuranoid C **103**	43
Walsura cochinchinensis	Walsucochin A **104** Walsucochin B **105**	44
Walsura yunnannensis	Walsurin **106** Isowalsuranolide **107** Walsuranolide **108** 11β-acetoxywalsuranolide **109** 20,22-dihydro-22-23-epoxywalsuranolide **110** 11β-hydroxydihydrocedrelone **111** 11β-acetoxydihydrocedrelone **112** 11β-hydroxycedrelone **113** Cedrelone **114**	45

Table 2.5 : Chemical constituents of Genus *Walsura.*



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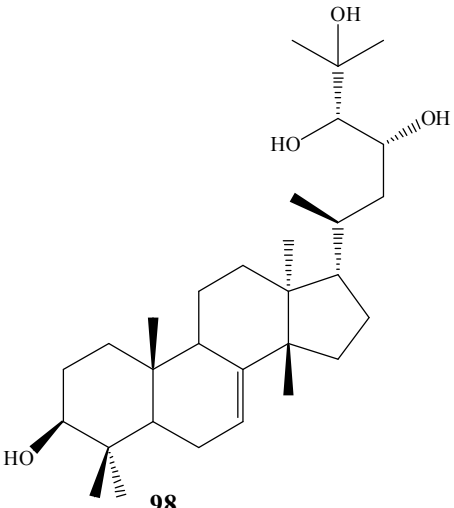
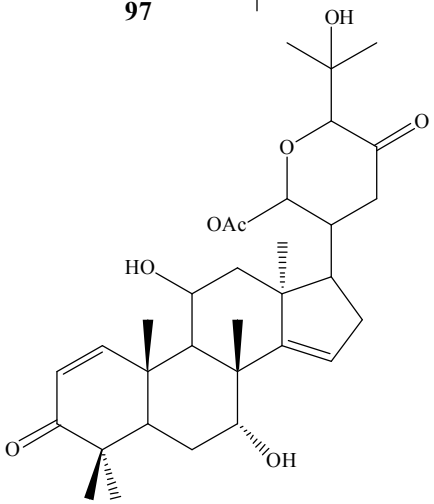


98



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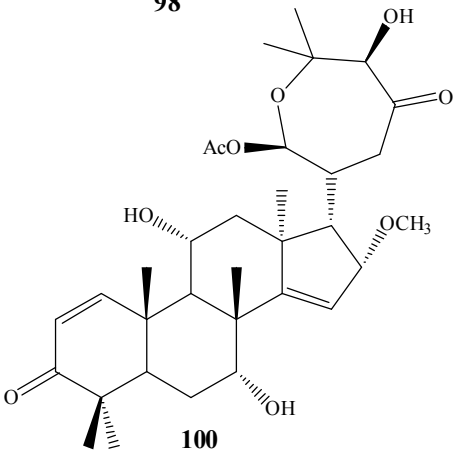

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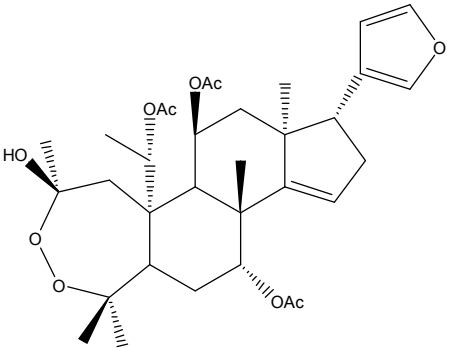

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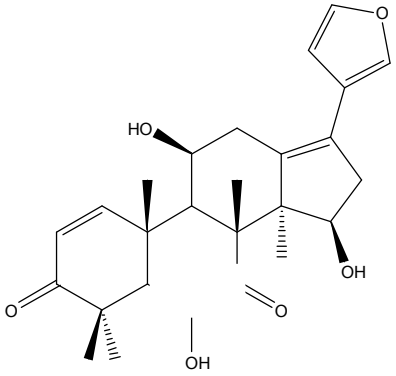

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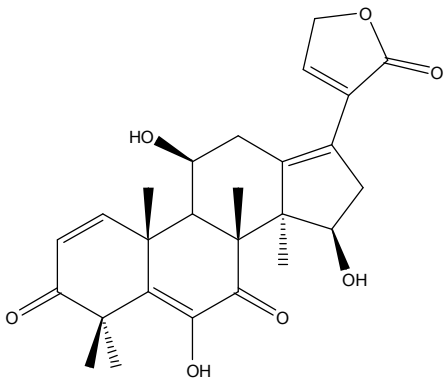

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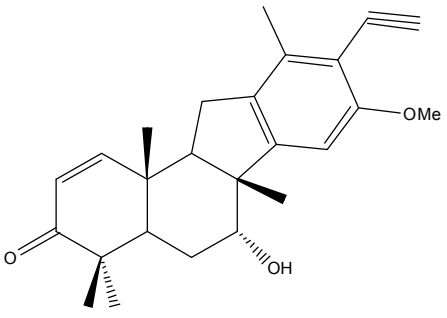

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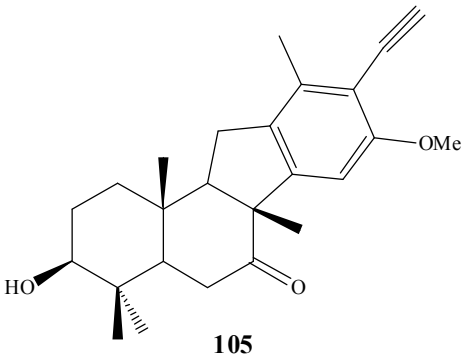

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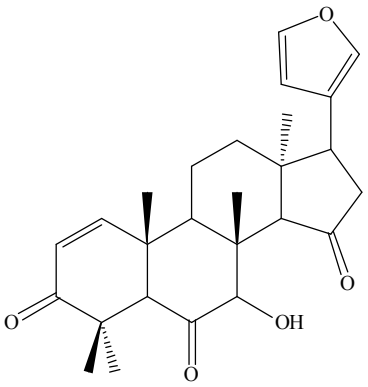

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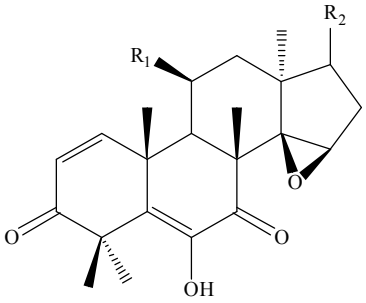
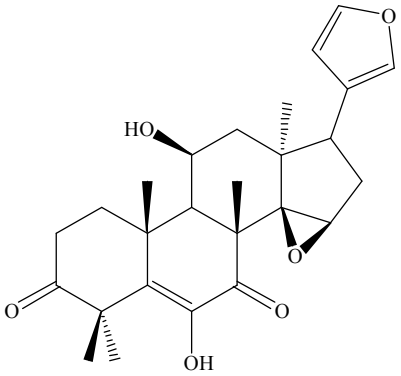
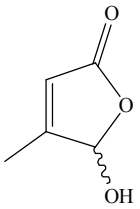


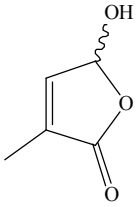


111

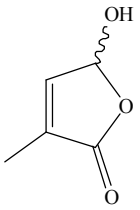
107 R1=H, R2=

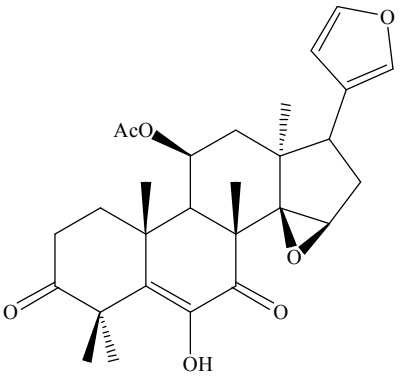


108 R1=H, R2=



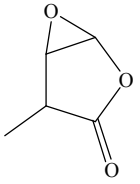
109 R1=OAc, R2=



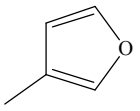


112

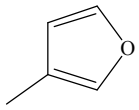
110 R1=H, R2=



113 R1=OH, R2=



114 R1=H, R2=



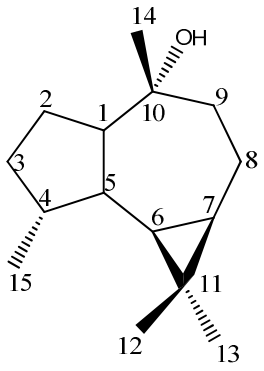
CHAPTER 3

RESULTS AND DISCUSSION

3.0 Introduction

Nine known compounds have been isolated from the bark of *Walsura pinnata* Hassk (KL 4571), which was collected from Kuala Lipis, Pahang. The isolation of pure compounds was accomplished through chromatographic methods; TLC and CC the structures of the isolated compounds were then elucidated by NMR (nuclear magnetic resonance), LCMS (liquid chromatography mass spectrometry) and IR (infrared) spectroscopy. Discussion on the structural elucidation of all the isolated compounds is represented in the following section of this chapter.

3.1 Compound A : ledol 115



Compound A, was isolated as gummy oil. The EIMS spectrum showed the molecular ion peak at *m/z* 204[M^+] corresponding to formula $C_{15}H_{26}O$. The IR spectrum of compound A displayed absorption bands at 3381 cm^{-1} due to the absorption of hydroxyl group.

The ^1H NMR spectrum (Figure 3.1.1) disclosed three tertiary methyl groups at δ_H 0.96;C$\underline{H}_3$-12, 1.02; C$\underline{H}_3$-13 and 1.12; C$\underline{H}_3$-14 and one doublet methyl group at δ 0.93;C$\underline{H}_3$-15 (*J*=6.9Hz). A very shielded *dd* δ_H0.31 (*J*=10.5, 5.9 Hz, H-6) and a *ddd* δ_H0.71 (*J*=15.1, 9.1, 5.9Hz, H-7) were observed and corresponds to the cyclopropane protons. These signals

are characteristic of aromadendrane-type sesquiterpenes. The spectral data, coupled with the degrees of unsaturation (three), suggested that compound A is a tricyclic sesquiterpene.

The ^{13}C NMR spectrum (Figure 3.1.2) combined with analysis of the DEPT spectrum (Figure 3.1.3) permitted differentiation of 15 resonances into two quaternary carbons, five methines, four methylenes, three tertiary methyls and one secondary methyl, of which were assigned to a sesquiterpene skeleton.

After direct ^{1}H and ^{13}C correlations were established from the HSQC spectrum (Figure 3.1.4), the gross structure of compound A was elucidated on the basis of the analysis of HMBC spectrum (Figure 3.1.5). The location of secondary methyl group between C$\underline{H}_3$-15 and C-3 was demonstrated by the HMBC correlations from C$\underline{H}_3$-15(δ_H0.93) to C-3(δ_C30.8) and C$\underline{H}_3$-15 to C-5 (δ_C38.5) indicated the connectivity between C-4 and C-5. The presence of a dimethylcyclopropyl group at C-6 and C-7 was exhibited by the HMBC correlations of C$\underline{H}_3$-13 (δ_H1.02) with C-7 (δ_C25.1) and C-11 (δ_C19.3) and of C$\underline{H}_3$-12 (δ_H0.96) with C-6 (δ_C23.4) and C-11(δ_C19.3). Finally, the HMBC correlations between C$\underline{H}_3$-14(δ_H1.12) and C-1(δ_C53.8), C-10(δ_C74.7), and C-9(δ_C39.2), confirming C1-C10-C-14 and C1-C10-C9 connectivities. The structure of compound A is thus confirmed to be ledol[46]. The complete assignments (Figure 3.1a) were made by 2D-NMR studies including HSQC and HMBC (Table 3.1)

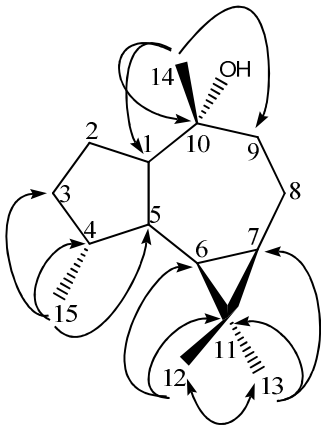


Figure 3.1a : Significant HMBC (→) interaction of Compound A

Table 3.1: ^{1}H, ^{13}C and HMBC Spectral Data of Compound A in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm (*J*,Hz)]	HMBC(H→C)
1	53.8	2.07(*m*)	
2	24.7	1.68(*m*)	14
		1.83(*m*)	
3	30.8	1.27(*m*)	15
		1.70(*m*)	
4	38,5	1.97(*m*)	1
5	40.8	1.77(*m*)	
6	23.4	0.31(*dd*, *J*=10.5, 9.1Hz)	5, 13
7	25.0	0.71(*ddd, J*=15.1, 9.1, 5.9Hz)	12
8	20.4	1.21(*m*)	12
		1.88(*m*)	
9	39.2	1.69(*m*)	10
		1.84(*m*)	
10	74.7		
11	19.3		
12	15.5	0.96(*s*)	7, 11, 13
13	28.7	1.02(*s*)	6, 11, 12
14	30.5	1.12(*s*)	1, 9, 10,
15	16.1	0.93(*d*, *J*=6.9Hz)	3, 4, 5

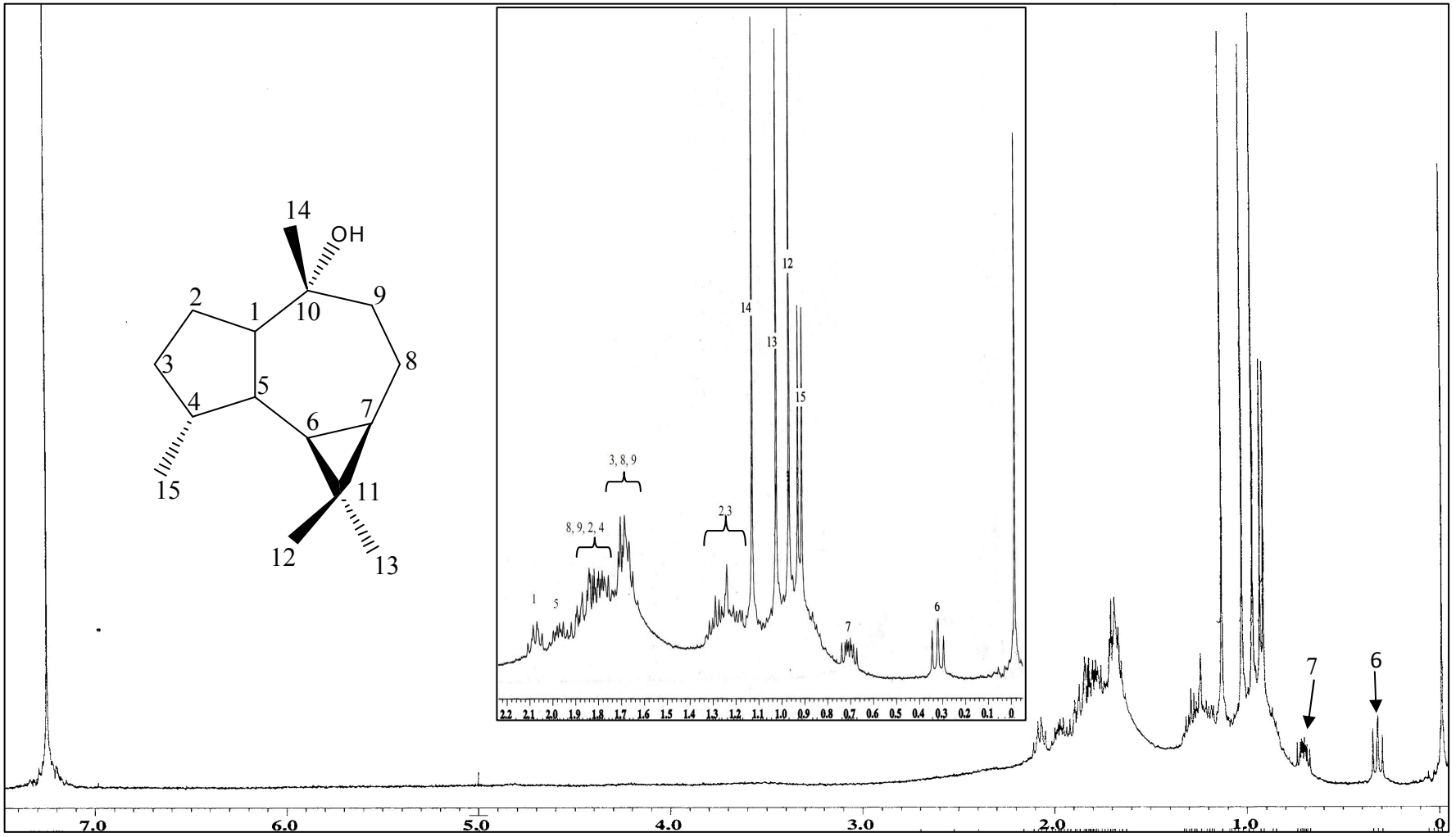


Figure 3.1.1: [1]HNMR Spectrum of Compound A

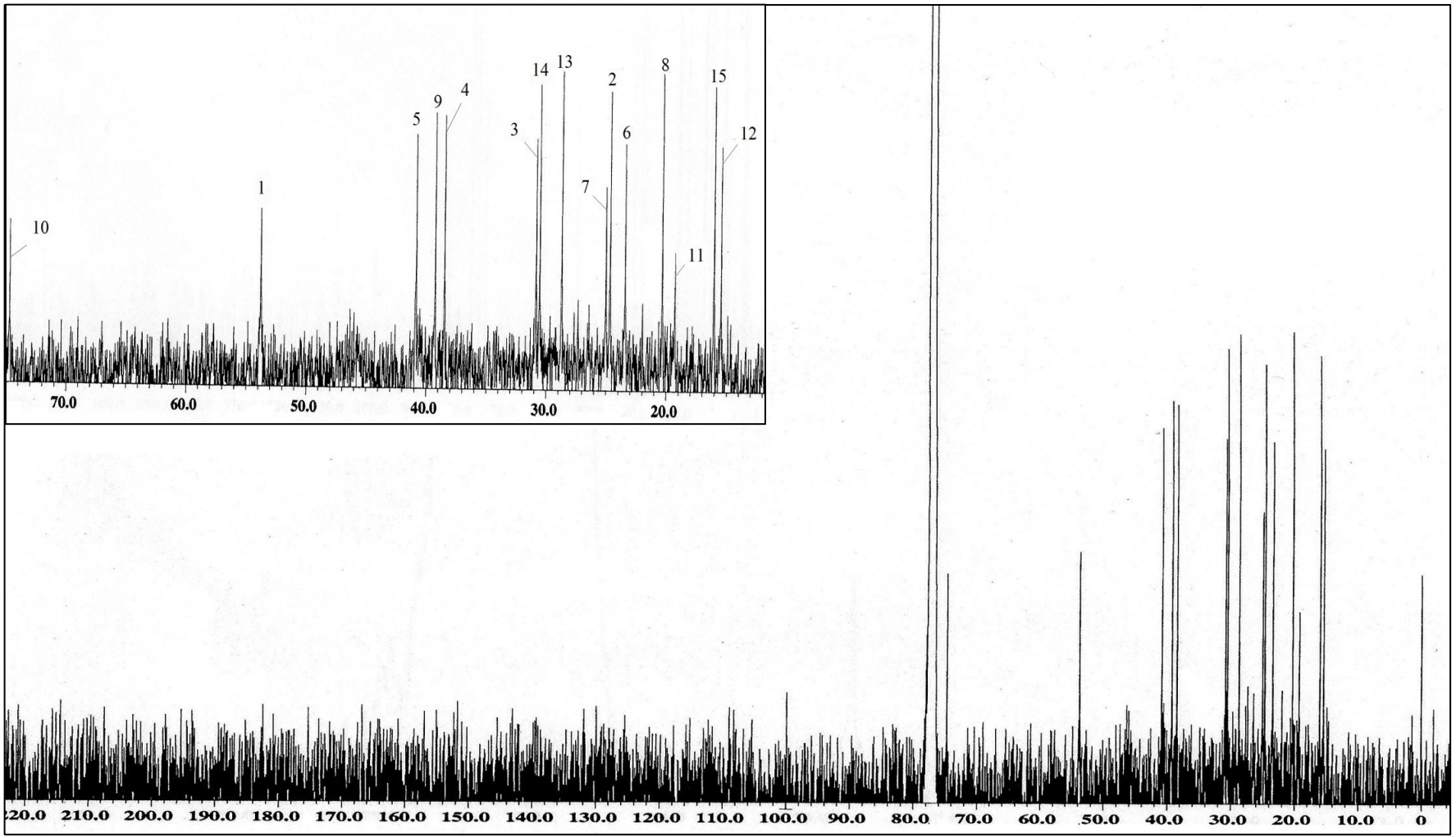


Figure 3.1.2: ^{13}CNMR Spectrum of Compound A

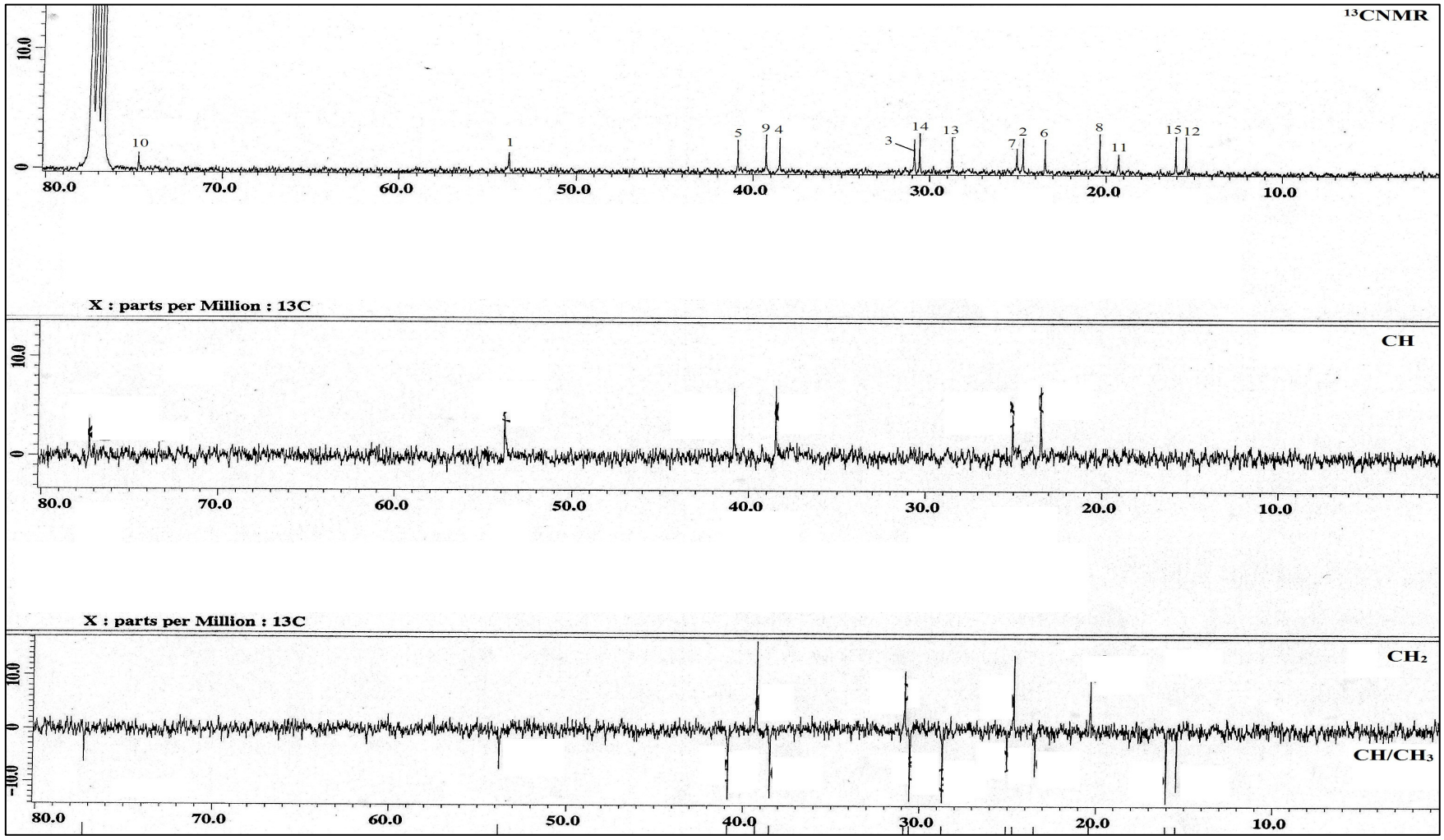


Figure 3.1.3: DEPT Spectrum of Compound A

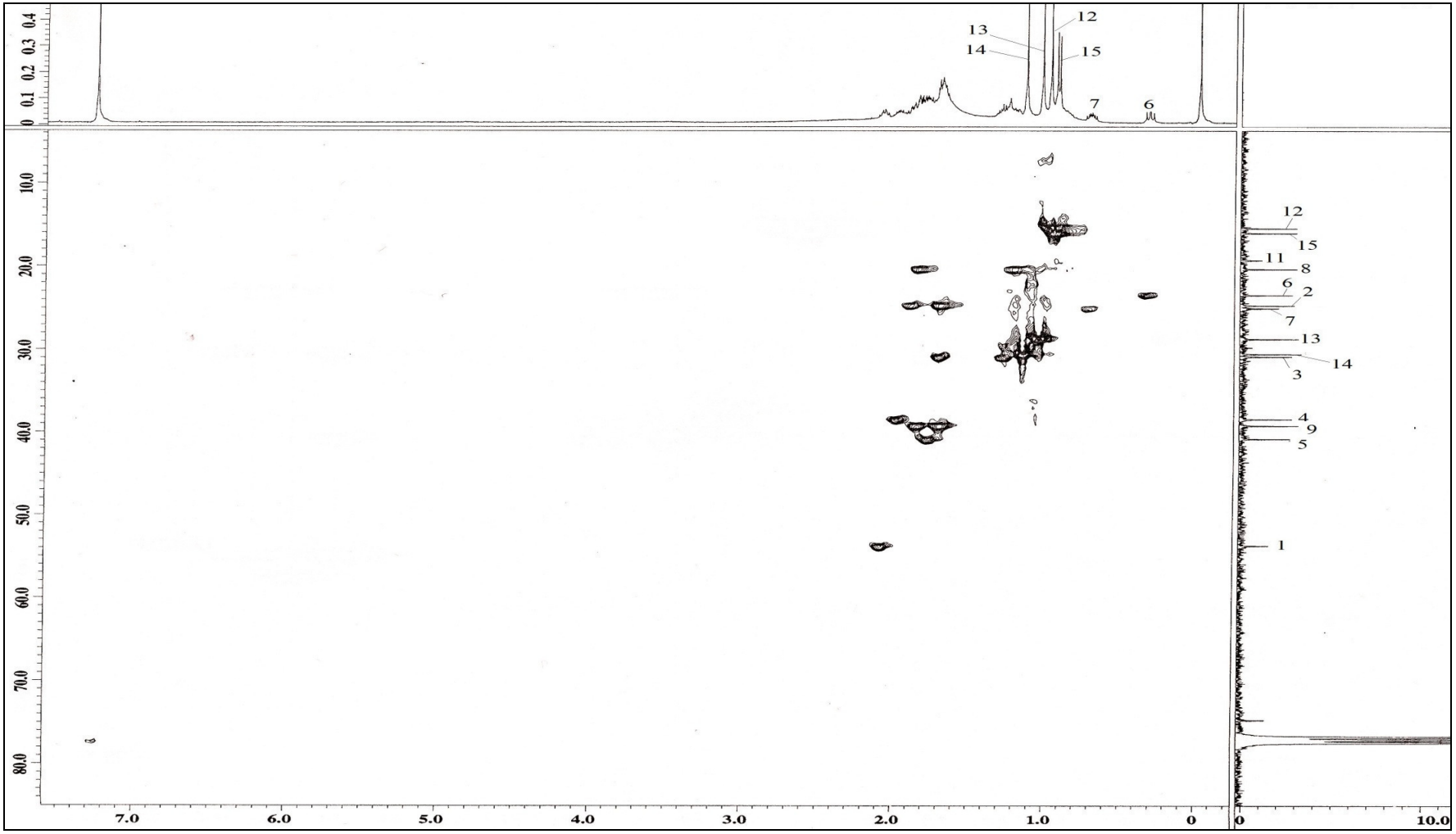


Figure 3.1.4: HSQC Spectrum of Compound A

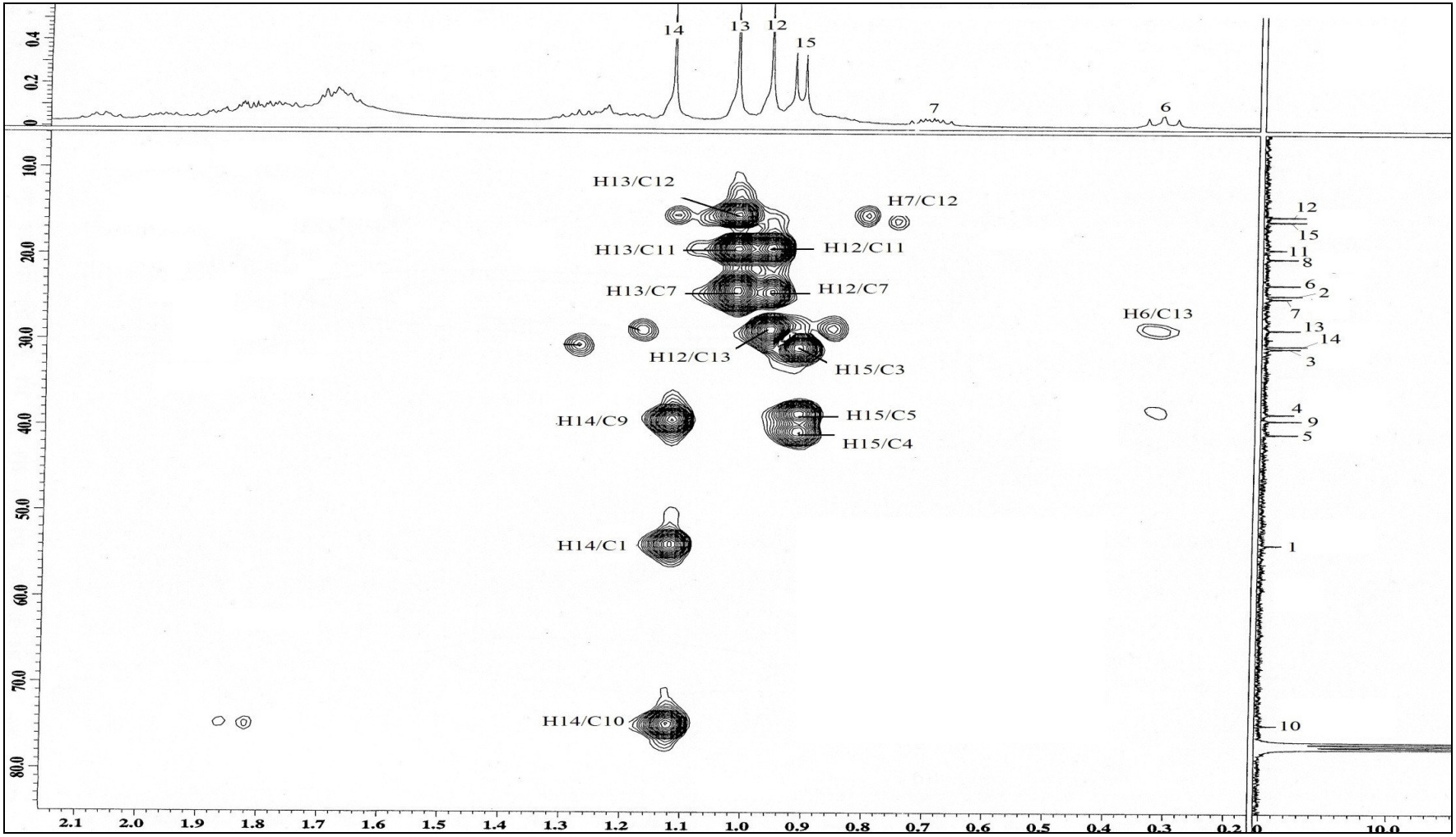
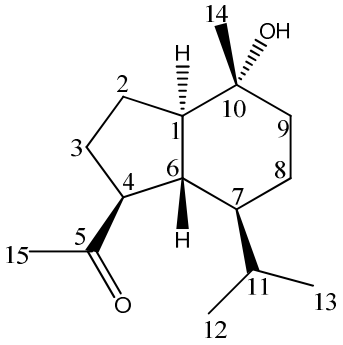


Figure 3.1.5: HMBC Spectrum of Compound A

3.2 Compound B : oplopanone 116



Compound B was obtained as colourless gummy oil. The IR spectrum showed absorption bands at 3376 and 1706cm^{-1} due to OH and C=O functional groups, respectively. The GCMS analysis gave the protonated molecular ion peak at *m/z* 238 corresponding to the molecular formula $C_{15}H_{26}O_2$, implying thus three degrees of unsaturation.

The ^{1}H NMR spectrum (Figure 3.2.1) and ^{13}C NMR spectrum (Figure 3.2.2) of compound B was characterized with a tertiary methyl group (δ_H1.19; δ_C20.4, C$\underline{H}_3$-14), one isopropyl group [(δ_H1.44; δ_C29.6, H-11), (δ_H0.68, *J*=6.8Hz; δ_C15.7, C$\underline{H}_3$-13), (δ_H0.88, *J*=6.8Hz; δ_C22.1, C$\underline{H}_3$-12)], a doublet of doublet of doublets at(δ_H2.65, *J*=14.6, 9.6, 5.4Hz; δ_C55.8, H-4) and one acetyl group [(δ_H2.18; δ_C29.6, C$\underline{H}_3$-15), (δ_C211.7, C-4)], which were similar with the literature[46,47].

The HMBC spectrum showed a correlation between (Figure 3.2.4) the singlet methyl proton signal of C$\underline{H}_3$-15(δ2.18), carbonyl carbon signal C-5(δ_C211.7) and C-4(δ_C55.8). Moreover the singlet methyl proton signal of C$\underline{H}_3$-14(δ_H1.19) showed cross peaks with the carbon signal of C-10(δ_C73.2) bearing hydroxyl group, methylene carbon signal of C-9(δ_C42.1) and methine carbon signal C-1(δ_C57.1). In addition, correlations were observed between the methyl protons C$\underline{H}_3$-12(δ_H0.88) and C$\underline{H}_3$-13(δ_H0.68) with the carbons C-11(δ_C29.6) and C-7(δ_C49.5). The remaining proton signals were assigned by the HSQC

spectrum (Figure 3.2.3). On the basis of these data, the proton and carbon chemical shifts of compound B were completely assigned as the cadinane type sesquiterpene oplopanone[47] in Table 3.2 and Figure 3.2a

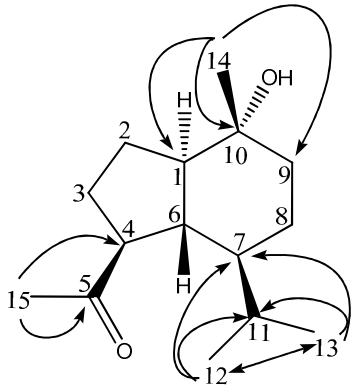


Figure 3.2a : Significant HMBC (→) interaction of Compound B

Table 3.2: ^{1}H, ^{13}C and HMBC Spectral Data of Compound B in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm (*J*,Hz)]	HMBC(H→C)
1	57.1	1.44(*m*)	
2	25.4	1.40(*m*)	4
		1.80(*m*)	
3	28.7	1.56(*m*)	4
		1.93(*m*)	
4	55.8	2.65(*ddd, J*=5.4, 9.6, 14.6Hz)	
5	211.7		
6	46.8	1.88(*m*)	
7	49.5	1.10(*m*)	13
8	23.1	1.08(*m*)	
		1.56(*m*)	
9	42.1	1.30(*m*)	14
		1.76(*m*)	
10	73.2		
11	29.6	1.44(*m*)	
12	22.1	0.88(*d*, *J*=6.8Hz)	7, 11, 13
13	15.7	0.68(*d*, *J*=6.8Hz)	7, 11, 12
14	20.4	1.19(*s*)	1, 9, 10
15	29.6	2.18(*s*)	4, 5

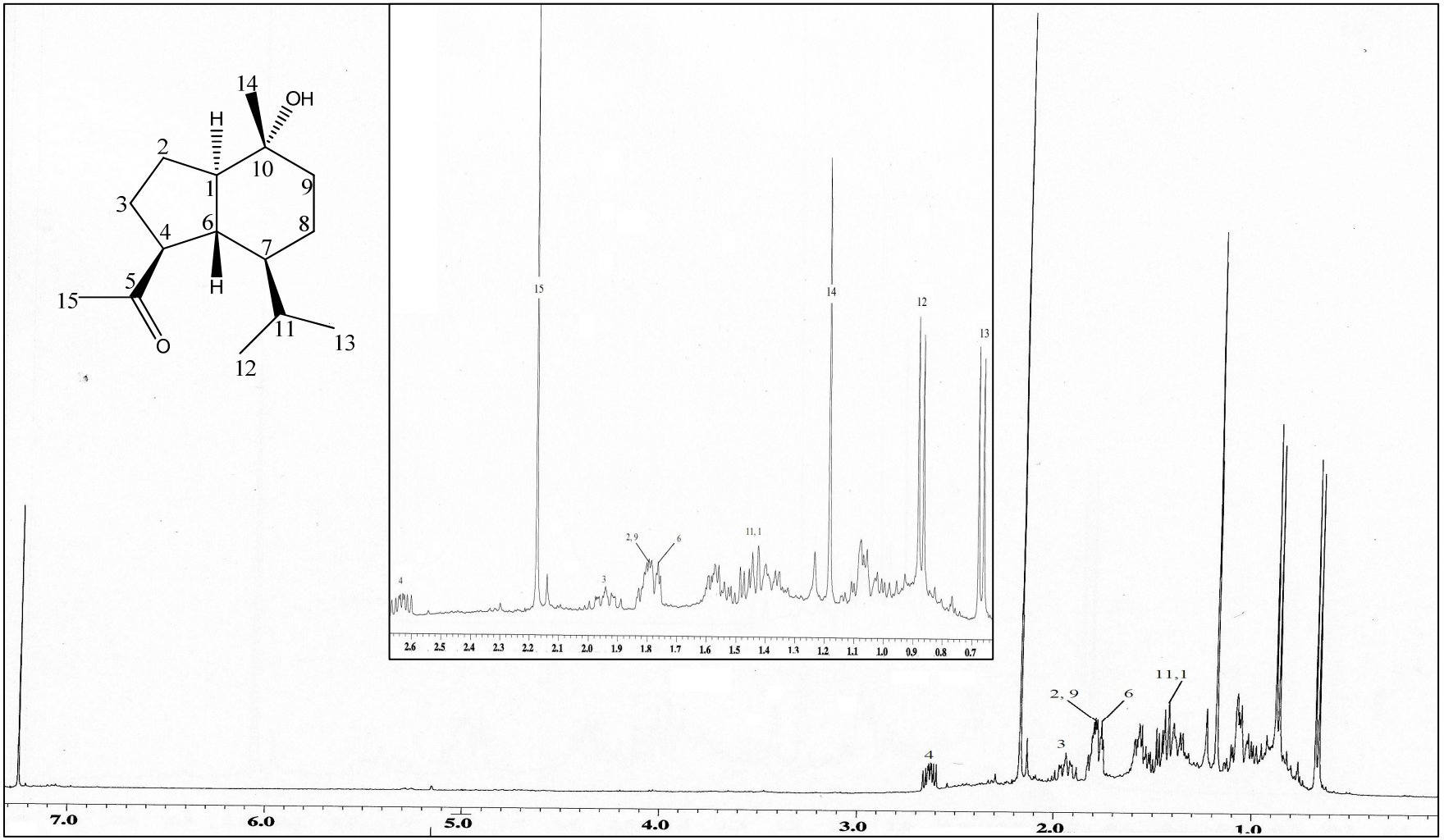


Figure 3.2.1: [1]HNMR Spectrum of Compound B

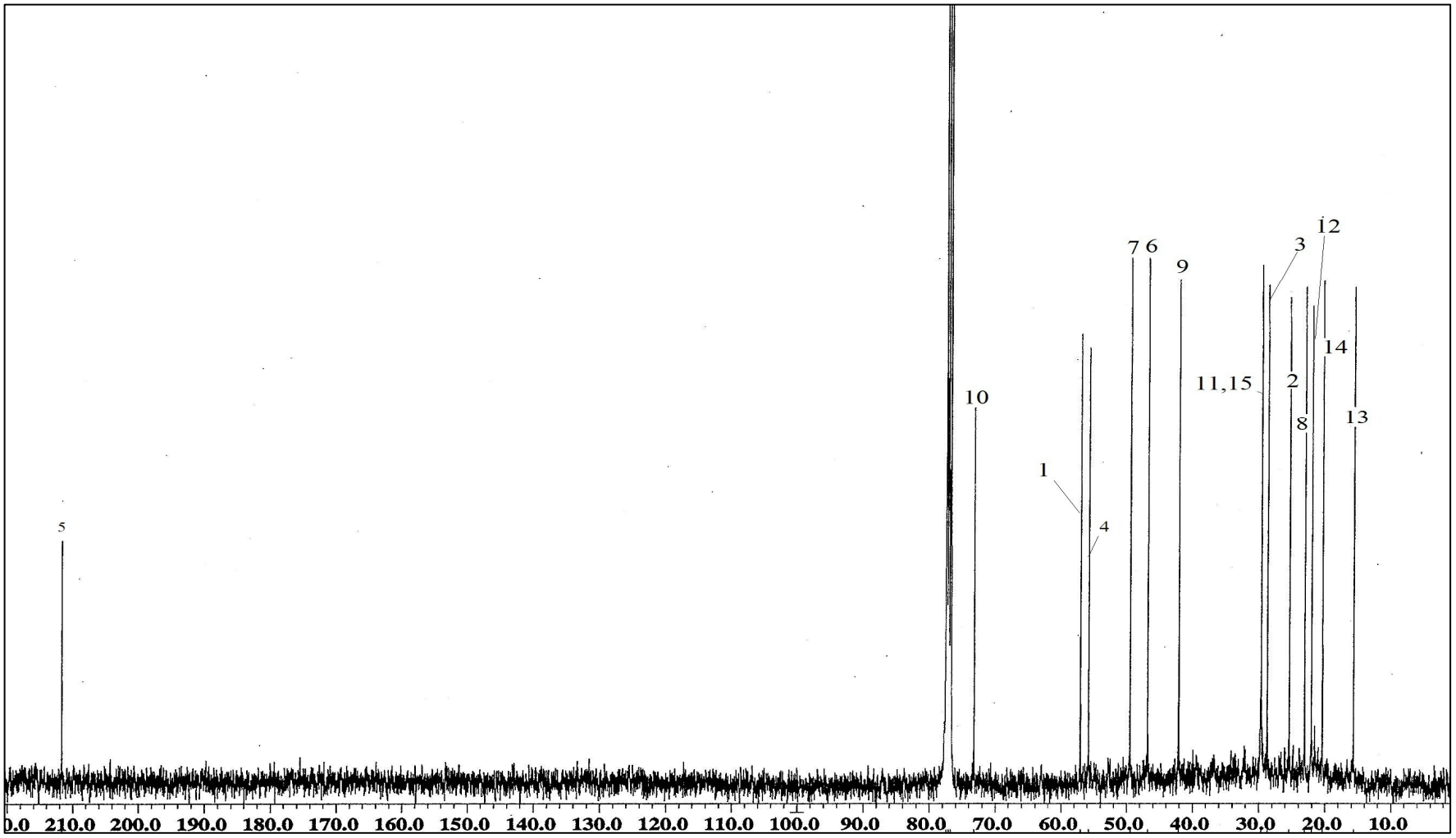


Figure 3.2.2: ^{13}CNMR Spectrum of Compound B

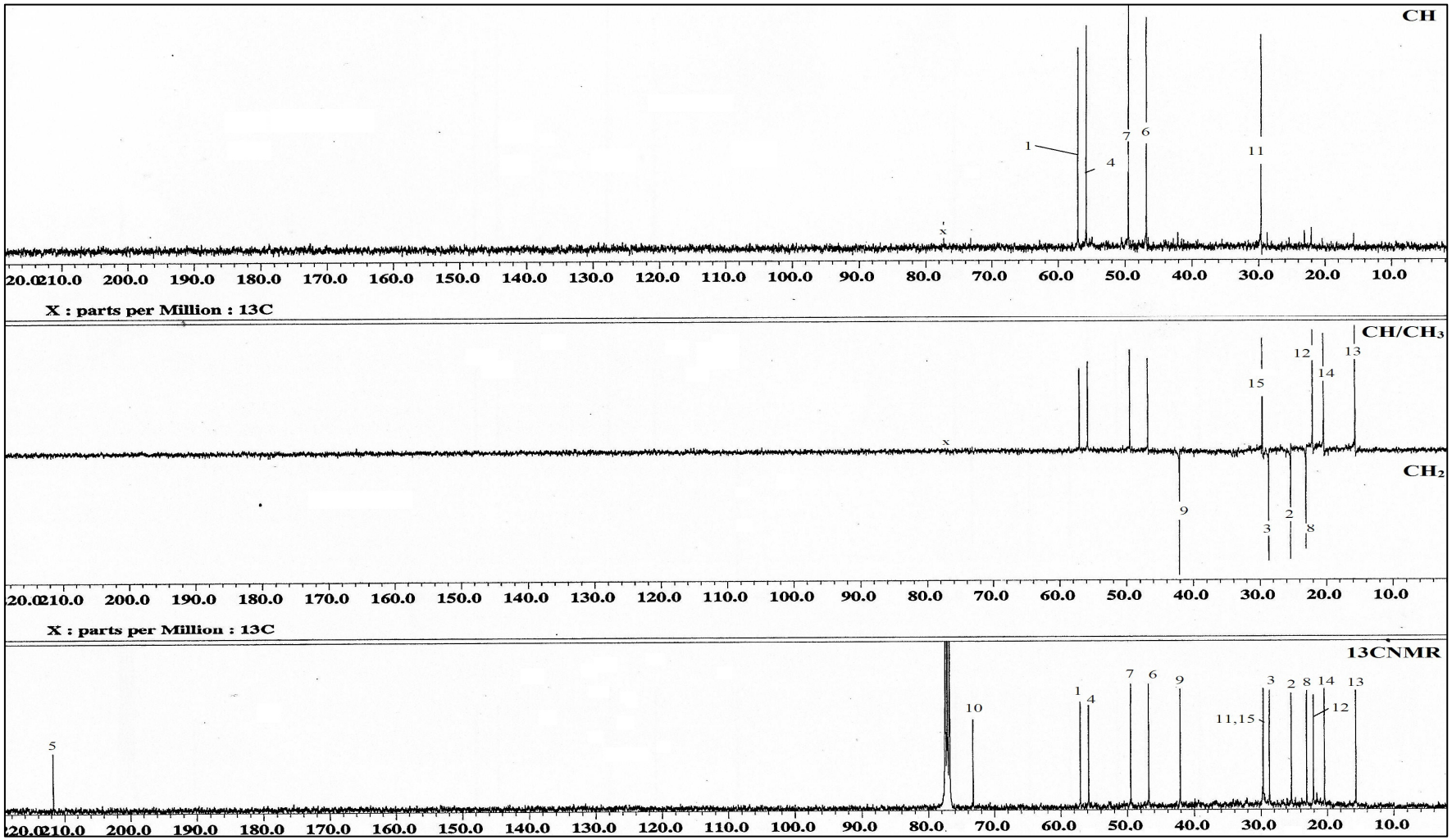


Figure 3.2.3:DEPT Spectrum of Compound B

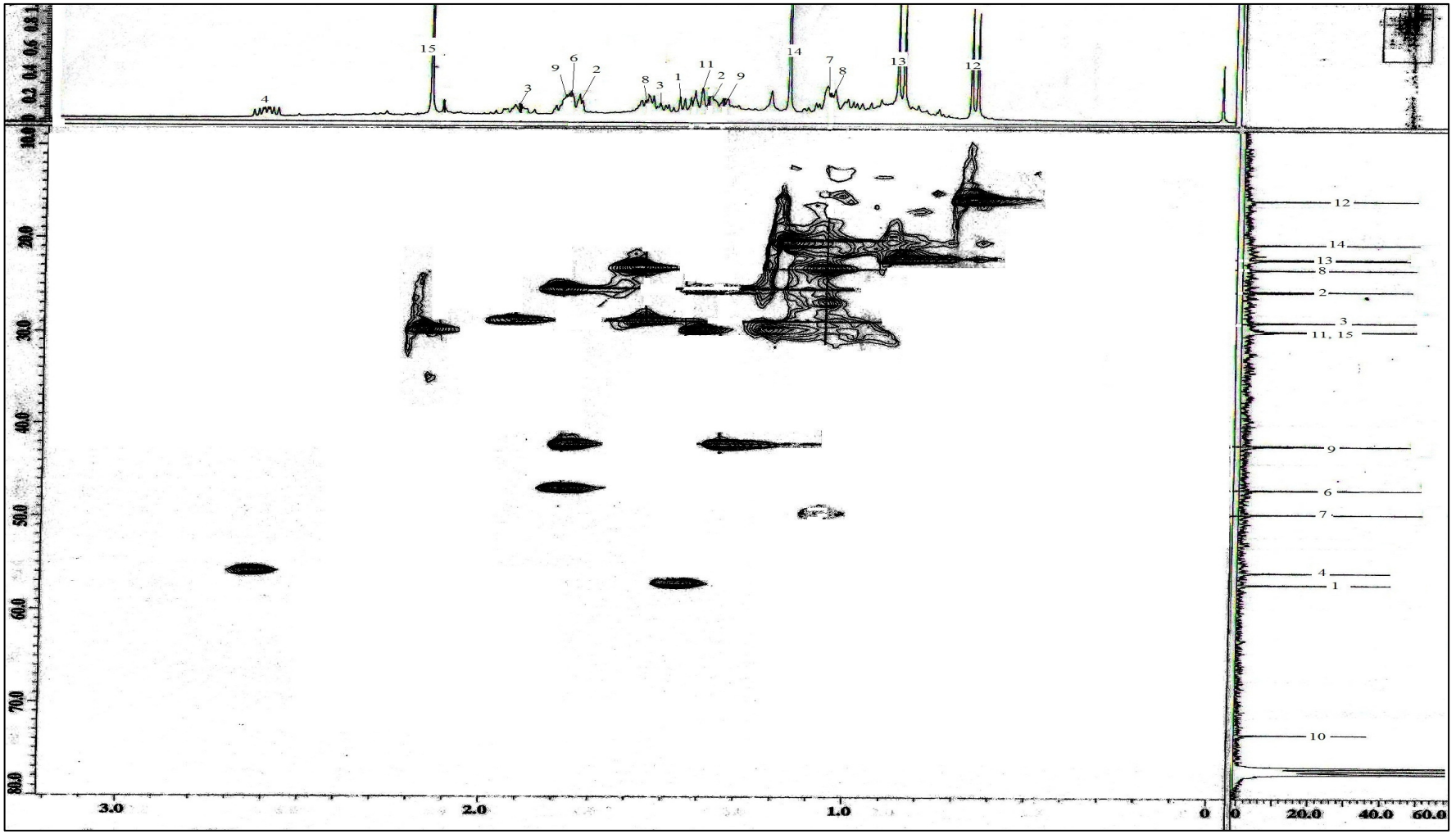


Figure 3.2.4:HSQC Spectrum of Compound B

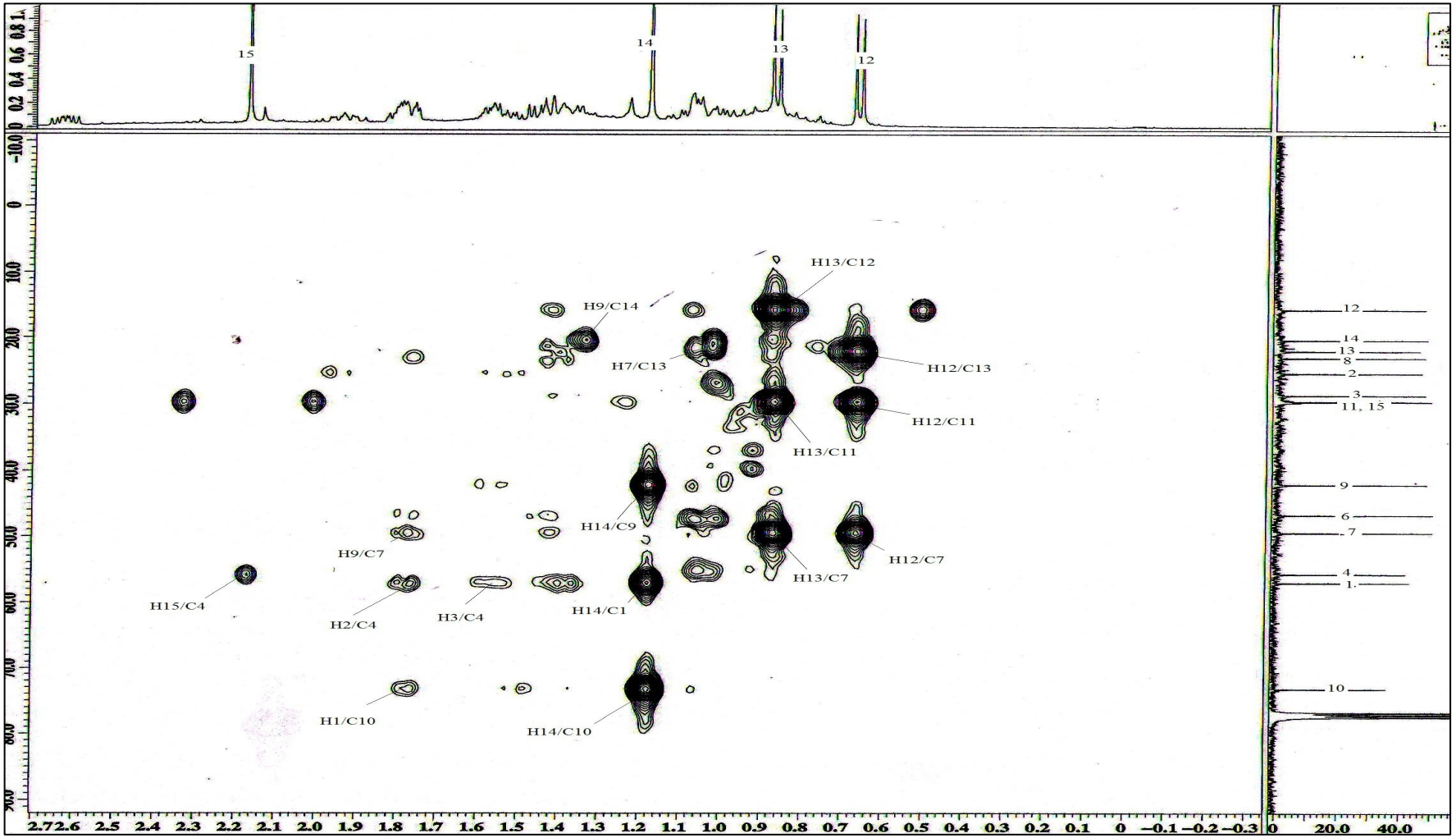
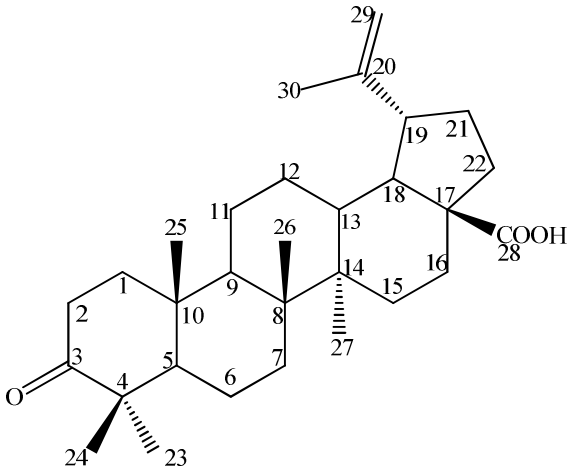


Figure 3.2.5:HMBC Spectrum of Compound B

Compound C : Betulonic acid 117



Compound C displayed a molecular ion peak at *m/z* 453.61 in the LCMS, and in agreement with the molecular formula of $C_{30}H_{46}O_3$. The IR spectrum of compound C showed absorption bands at 3072-2870 cm^{-1}(COOH) and 1694 cm^{-1} (ketone C=O).

The ^{1}H NMR spectrum (Figure 3.3.1) of compound C exhibited five singlets of five tertiary methyl groups at δ_H0.90, 0.95, 0.96, 0.99 and 1.04 ppm corresponding to C$\underline{H}_3$-25, C$\underline{H}_3$-26, C$\underline{H}_3$-27, C$\underline{H}_3$-24 and C$\underline{H}_3$-23. Futher signals were observed at δ_H1.67 (C$\underline{H}_3$-30), 4.72ppm (Hb-29), and 4.59(Ha-29), indicating the presence of an isopropenyl group in the structure of compound C that confirmed the characteristic features for triterpenes of the lupane type.

The ^{13}C NMR spectrum (Figure 3.3.2) displayed resonances at δ 218.4 and 181.5 which can be assigned to C-3, and C-28, respectively. The resonances at δ 19.5(C-30), 109.9(C-29) and 150.5(C-20) may be attributed to the isopropenyl group. This signal correlated with the signal at δ_H2.97(H-19) in the HMBC spectrum (Figure 3.3.5). The results indicated that compound C is the lupane type triterpene, having a 3-keto group.

In addition to ^{13}C NMR spectrum and DEPT spectrum (Figure 3.3.3) revealed the presence of six methyls, eleven methylenes, five methines and eight quaternary carbons which provided further evidence for a pentacyclic triterpene skeleton. The $\Delta^{20,29}$-functionality of a lupane skeleton was inferred for this compound from the resonances of sp^2 carbons at C-29 (secondary carbon signal deduced by DEPT pulse sequence) at δ 109.9 and C-20 (quaternary carbon) at δ 150.5. The analysis of the NMR spectrum (Figure 3.3a) and complete assignment of all the protons and carbons (Table 3.3) by 1D and 2D NMR spectra confirmed that compound C is betulonic[48].

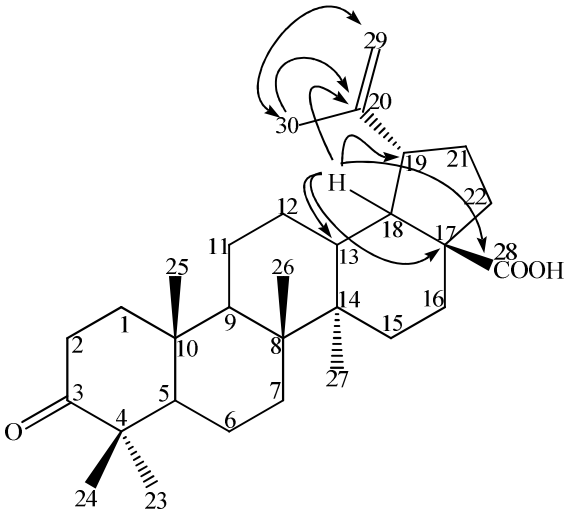


Figure 3.3a : Significant HMBC (→) interaction of Compound C

Table 3.3: ^{1}H, ^{13}C and HMBC Spectral Data of Compound C in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*,Hz)]	HMBC(H→C)
1	39.8	1.31(*m*)	2, 3, 5, 6, 25
		1.87(*m*)	
2	34.3	2.40(*m*)	1, 3
3	218.4		
4	47.5		
5	55.1	1.30(*m*)	6
6	19.8	1.50(*m*)	
7	33.8	2.20(*m*)	
		2.27(*m*)	
8	40.8		
9	50.0	1.34(*m*)	8, 10,
10	37.2		
11	21.5	1.40(*m*)	
12	25.7	2.98(*m*)	
13	38.7	2.24(*m*)	
14	42.7		
15	29.8		28
16	32.3	1.50(*m*)	
		1.96(*m*)	
17	56.5		
18	49.4	1.60(*m*)	13, 17, 19 , 20, 28
19	47.0	2.97(*m*)	
20	150.5		
21	30.7	1.40(*m*)	18, 28
		2.00(*m*)	
22	37.1		
23	26.8	1.04(*s*)	3, 4, 5, 24
24	21.2	0.99(*s*)	3, 4, 5, 23
25	16.0	0.90(*s*)	5, 9
26	16.1	0.95(*s*)	7, 9, 14
27	14.8	0.96(*s*)	8, 15,
28	181.5		
29	109.9	4.59(*br s*)	21, 30
		4.72(*br s*)	
30	19.5	1.67(*s*)	20, 29

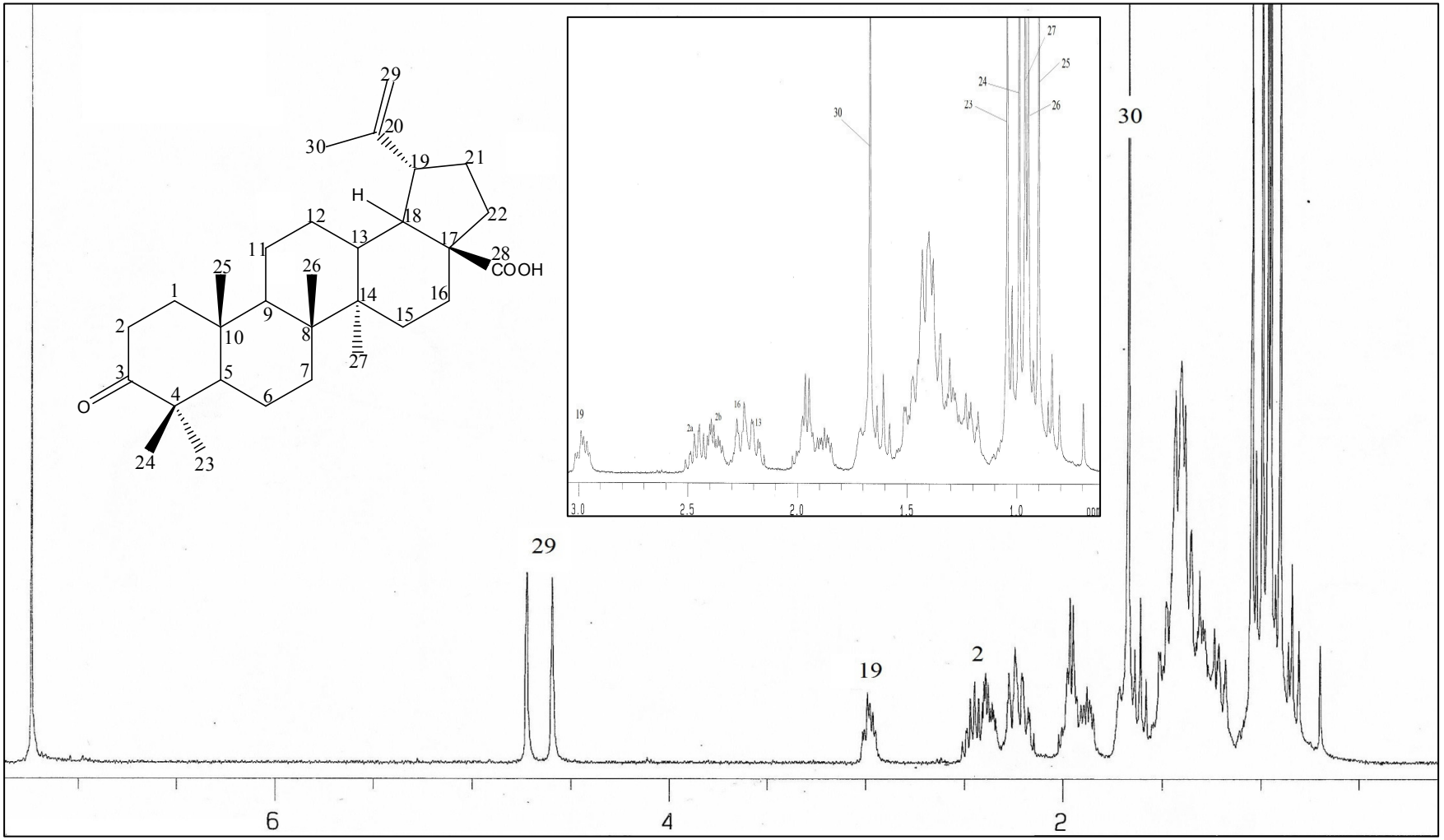


Figure 3.3.1: ^{1}HNMR Spectrum of Compound C

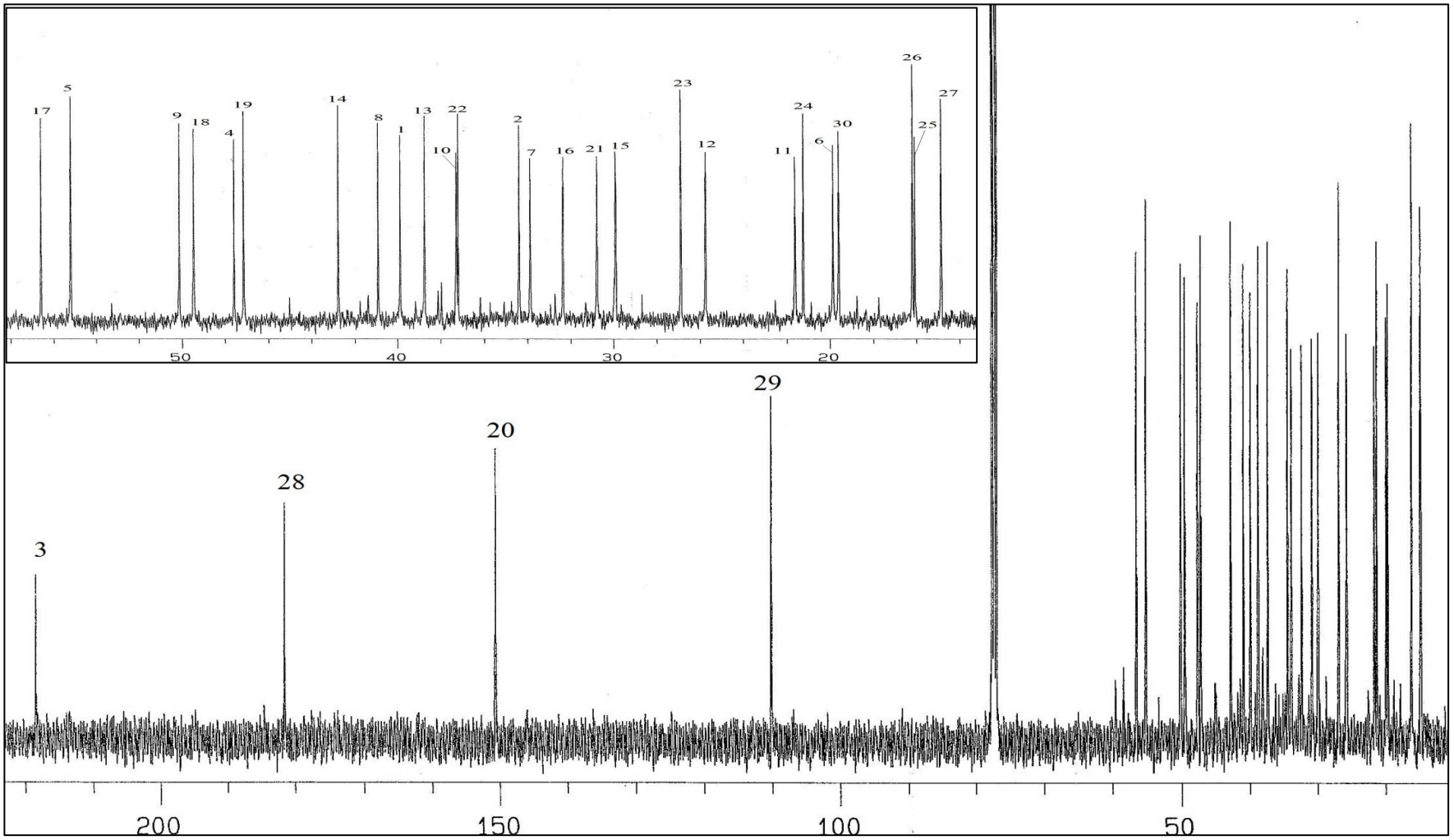


Figure 3.3.2: ^{13}CNMR Spectrum of Compound C

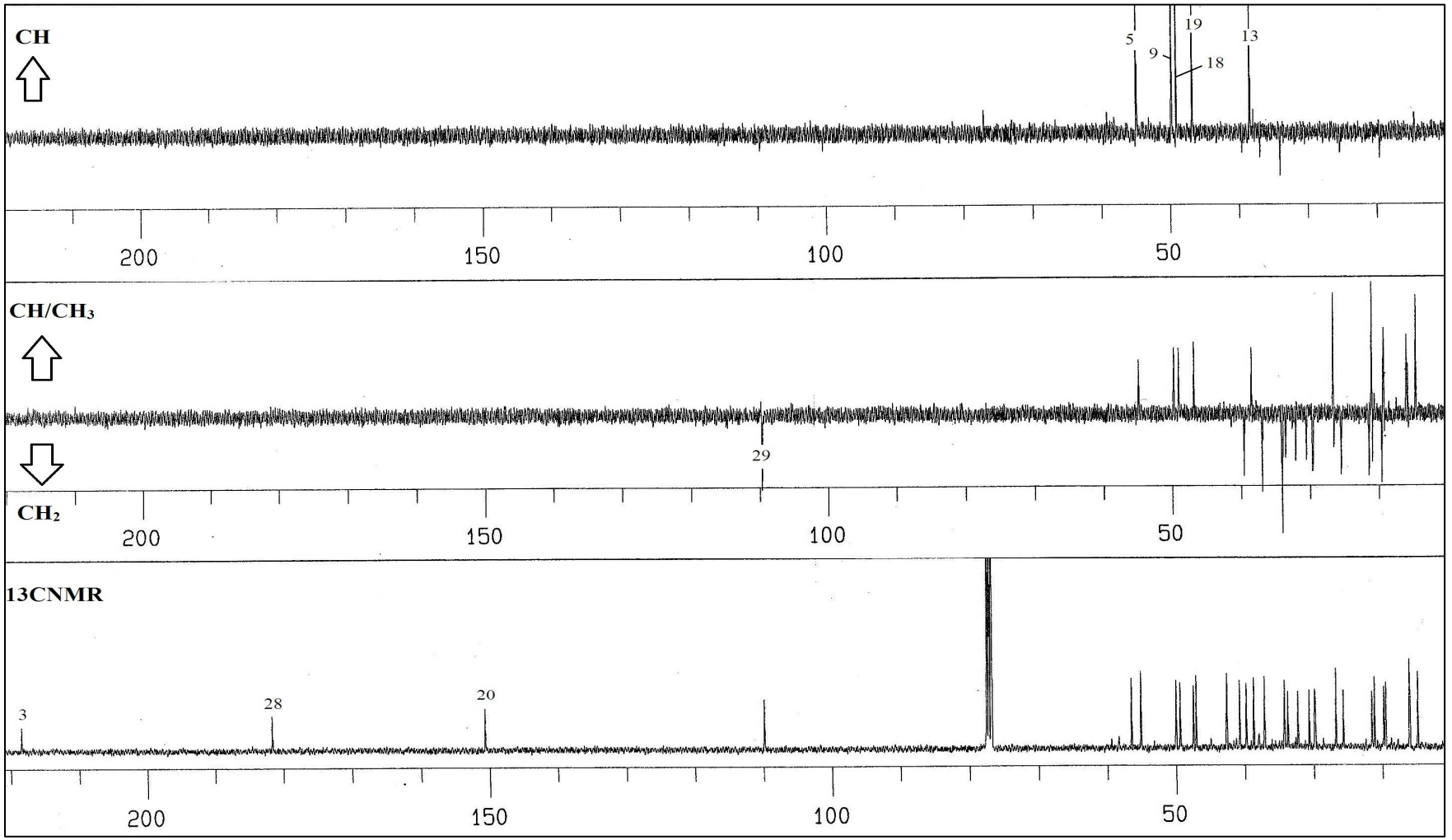


Figure 3.3.3: DEPT Spectrum of Compound C

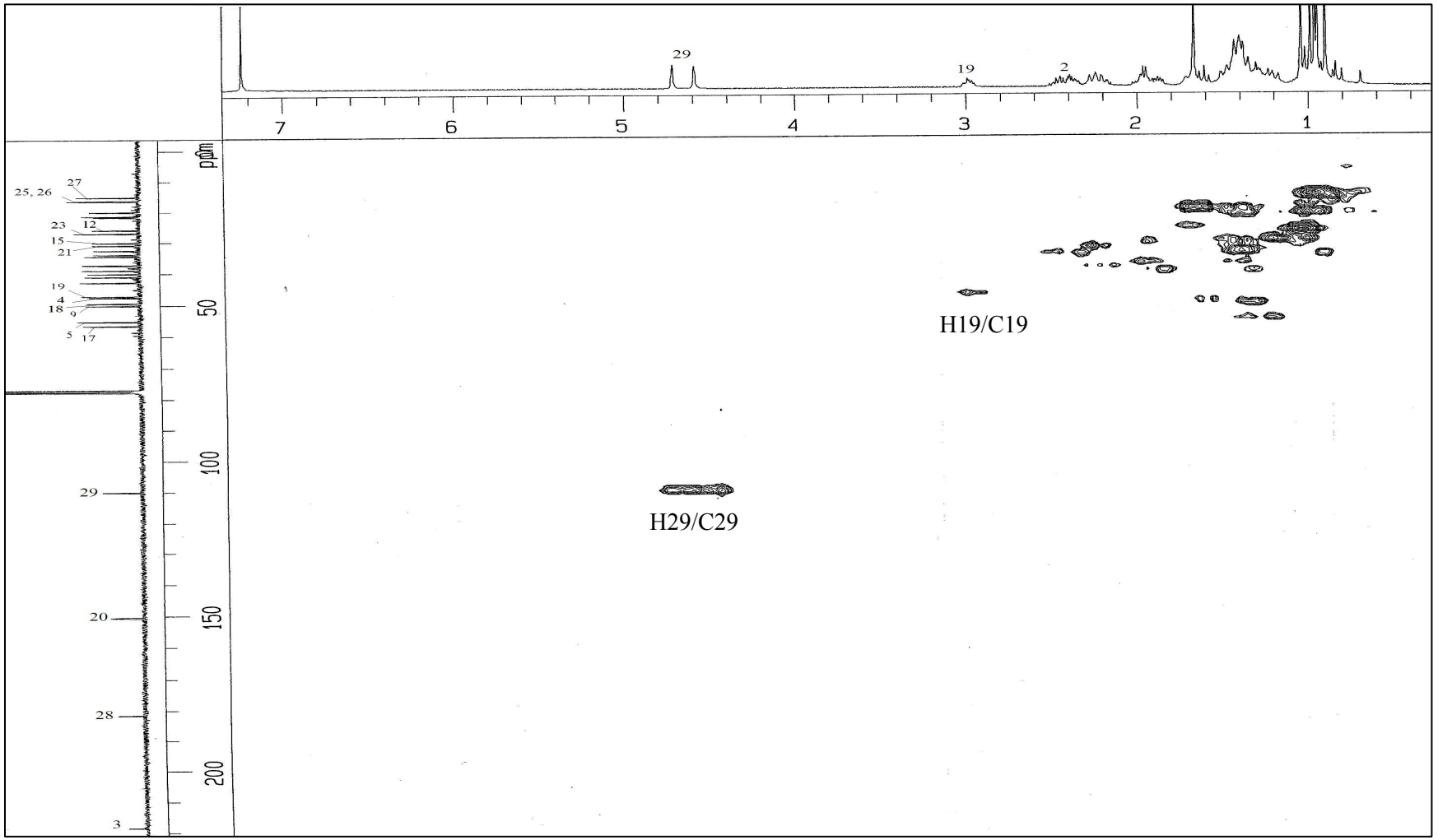


Figure 3.3.4: HSQC Spectrum of Compound C

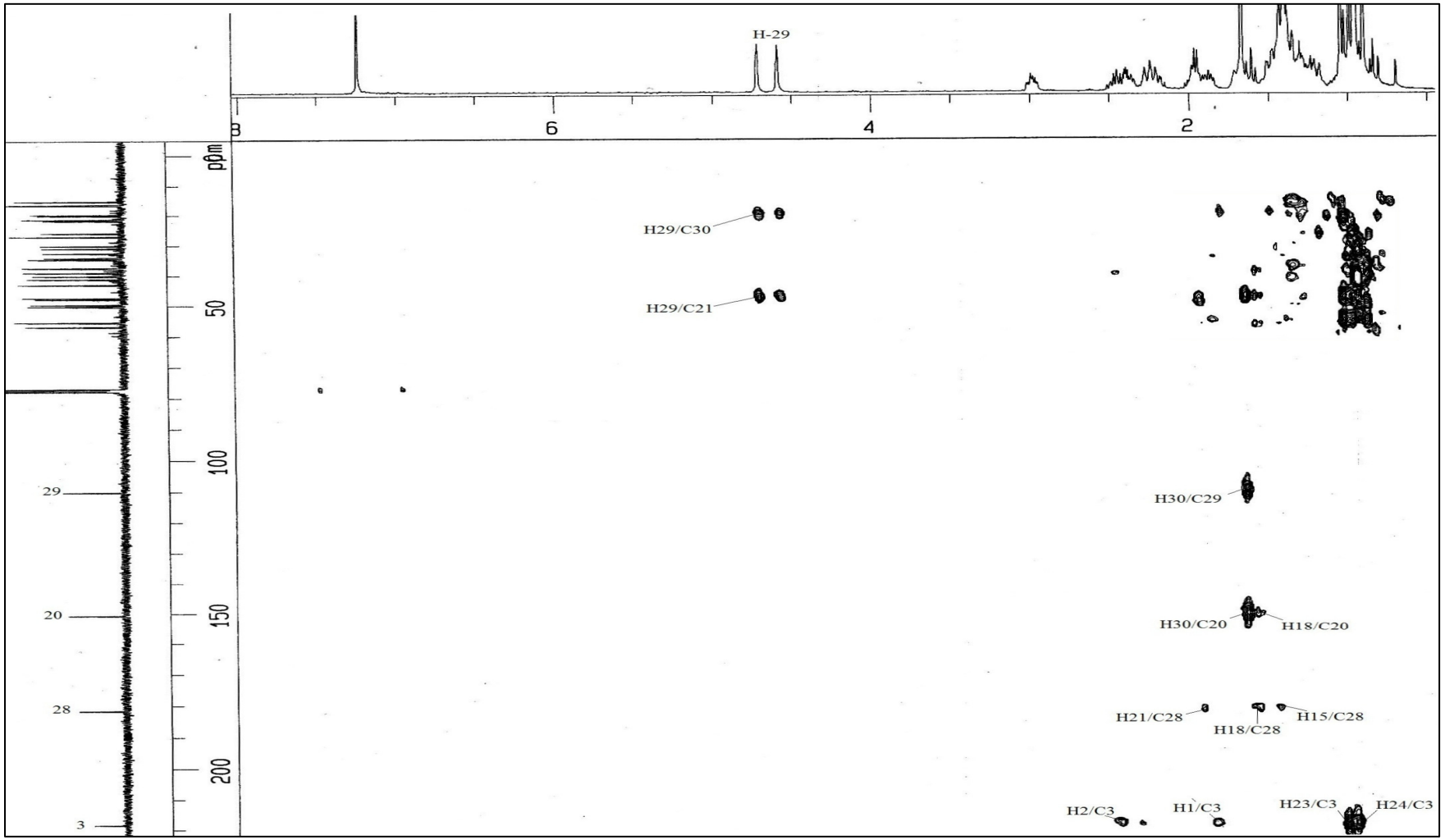
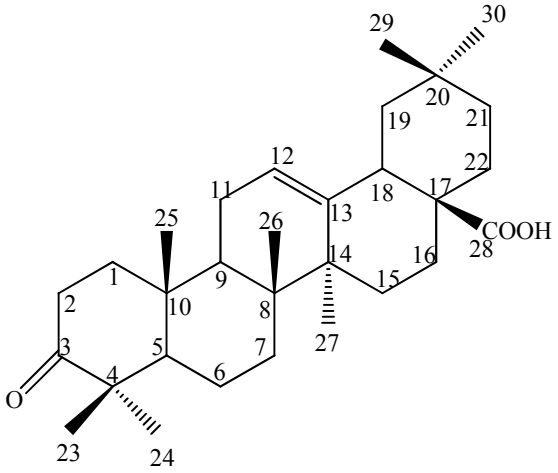


Figure 3.3.5: HMBC Spectrum of Compound C

3.4 Compound D : Oleanonic acid 118



Compound D was isolated as white crystals with mp 178°C. The ESI-LCMS showed an $(M)^+$ ion peak at 454 ,supported the molecular formula of $C_{30}H_{47}O_3$ which indicated eight degrees of unsaturation. The IR spectrum of compound D showed an absorption bands at 2947 (*br*, hydroxyl acid, OH) and 1696 cm^{-1} (ketone, C=O).

The ^1H NMR spectrum (Figure 3.4.1) exhibited seven methyl singlets (δ_H0.81;C$\underline{H}_3$-26, 0.90;C$\underline{H}_3$-29, 0.93;C$\underline{H}_3$-30, 1.03;C$\underline{H}_3$-24, 1.04;C$\underline{H}_3$-25, 1.08;C$\underline{H}_3$-23, and 1.11;C$\underline{H}_3$-27), one olefinic methine (δ_H5.30, br s, H-12) and a characteristic methine proton at δ2.83 (dd, *J*=13.6, 3.6Hz, H-18)[49].

The ^{13}C NMR (Figure 3.4.2) displayed 30 carbons signals including seven methyls (δ_C 15.0;C-25, 16.9;C-26, 21.4;C-24, 23.5;C-30, 25.8;C-27, 26.4;C-23 and 33.0;C-29) and two olefinic carbons (δ_C122.3 and 143.6), which were typical of the double bond at C-12 and C-13 of oleanane type triterpenes. Signal at δ_C182.9(C-28) indicated the presence of carboxylic group. In addition, chemical shifts of C-17, C-18, C-19, C-20 and C-22 at δ_C 46.5, 41.0, 45.8, 30.6 and 32.3 respectively suggested that compound D belongs to the β-amyrin series of pentacyclic[49].

These observations were further confirmed by the correlations detected in the HMBC spectrum (Figure 3.4.4) between H-18(δ_H 2.83) and C-11(δ_C 22.9), C-19(δ_C 45.8), C17(δ_C 46.5), C-12(δ_C 122.3), C-13(δ_C 143.6) and C-28(δ_C 183.0), giving an evidence that the double bond group at C-12 and C-13, and carboxyl group at C-28. Detail analysis of HMBC spectrum allowed the assignment of all the ^{1}H NMR and ^{13}C NMR signals, confirming the structure of compound D as the oleanane type triterpene called as oleanonic acid[50] (Figure 3.4a).

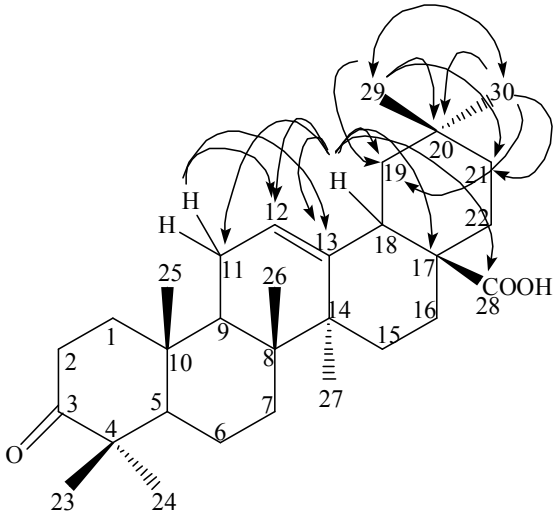


Figure 3.4a : Significant HMBC (→) interaction of Compound D

Table 3.4: ^{1}H, ^{13}C and HMBC Spectral Data of Compound D in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*,Hz)]	HMBC(H→C)
1	39.1	1.27(*m*)	3
2	34.1	2.45(*m*)	3
		2.52(*m*)	
3	217.7		
4	47.4		
5	55.3	1.18(*m*)	23, 24, 25
6	19.5	1.24(*m*)	
		1.38(*m*)	
7	32.4	1.59(*m*)	26, 27
8	39.3		
9	46.9	1.49(*m*)	
10	36.8		
11	22.9	1.84(*m*)	12, 13
		1.78(*m*)	
12	122.3	5.30(*br s*)	14, 16, 17
13	143.6		
14	41.7		
15	27.6	1.51(*m*)	13, 16
		1.58(*m*)	
16	23.5	1.35(*m*)	15
		1.56(*m*)	
17	46.5		
18	41.0	2.83(*dd*, *J*=13.9, 3.6Hz)	11, 12, 13, 17, 19, 28
19	45.8	1.72(*m*)	18
20	30.7		
21	33.8	2.29(*m*)	
		2.52(*m*)	
22	32.2	1.37(*m*)	18
		1.42(*m*)	
23	26.4	1.08(*s*)	3, 4, 5 ,24
24	21.4	1.03(*s*)	3, 4, 5 ,23
25	15.0	1.04(*s*)	10
26	16.9	0.81(*s*)	8, 9, 14
27	25.8	1.11(*s*)	8, 14
28	183.0		
29	33.0	0.90(*s*)	19, 20, 21, 30
30	23.5	0.93(*s*)	19, 20, 21, 29

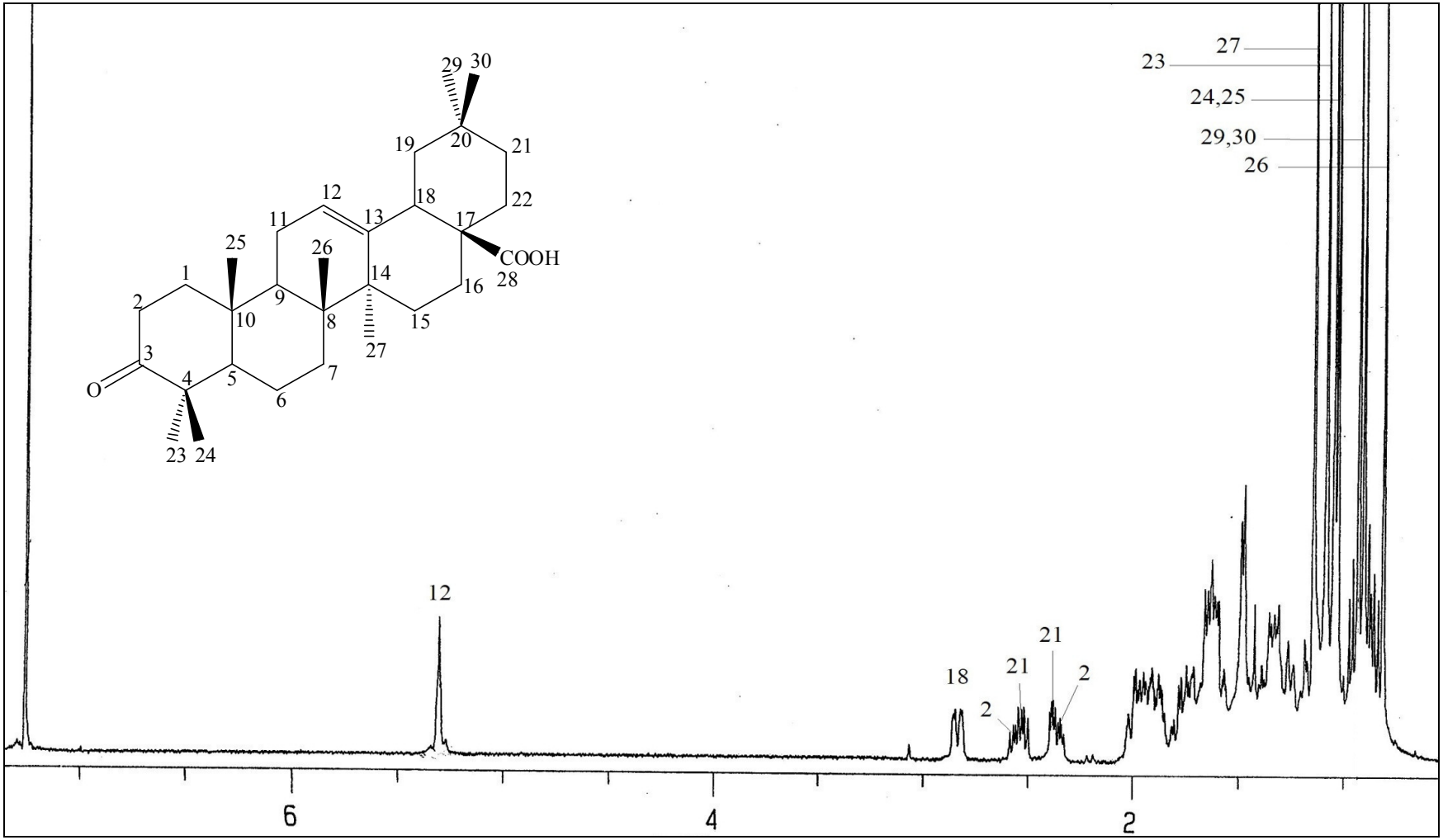


Figure 3.4.1: [1]HNMR Spectrum of Compound D

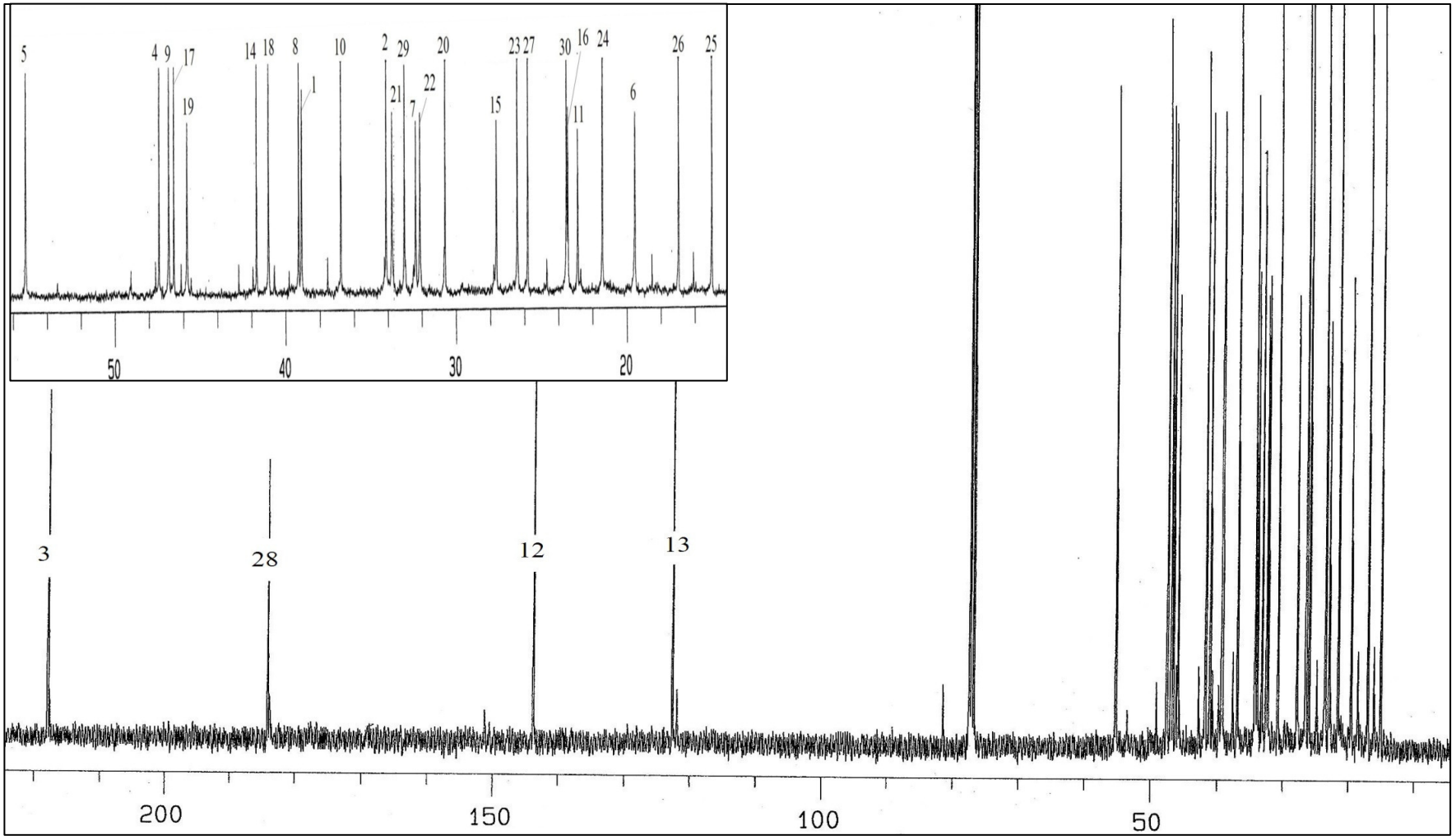


Figure 3.4.2: ^{13}CNMR Spectrum of Compound D

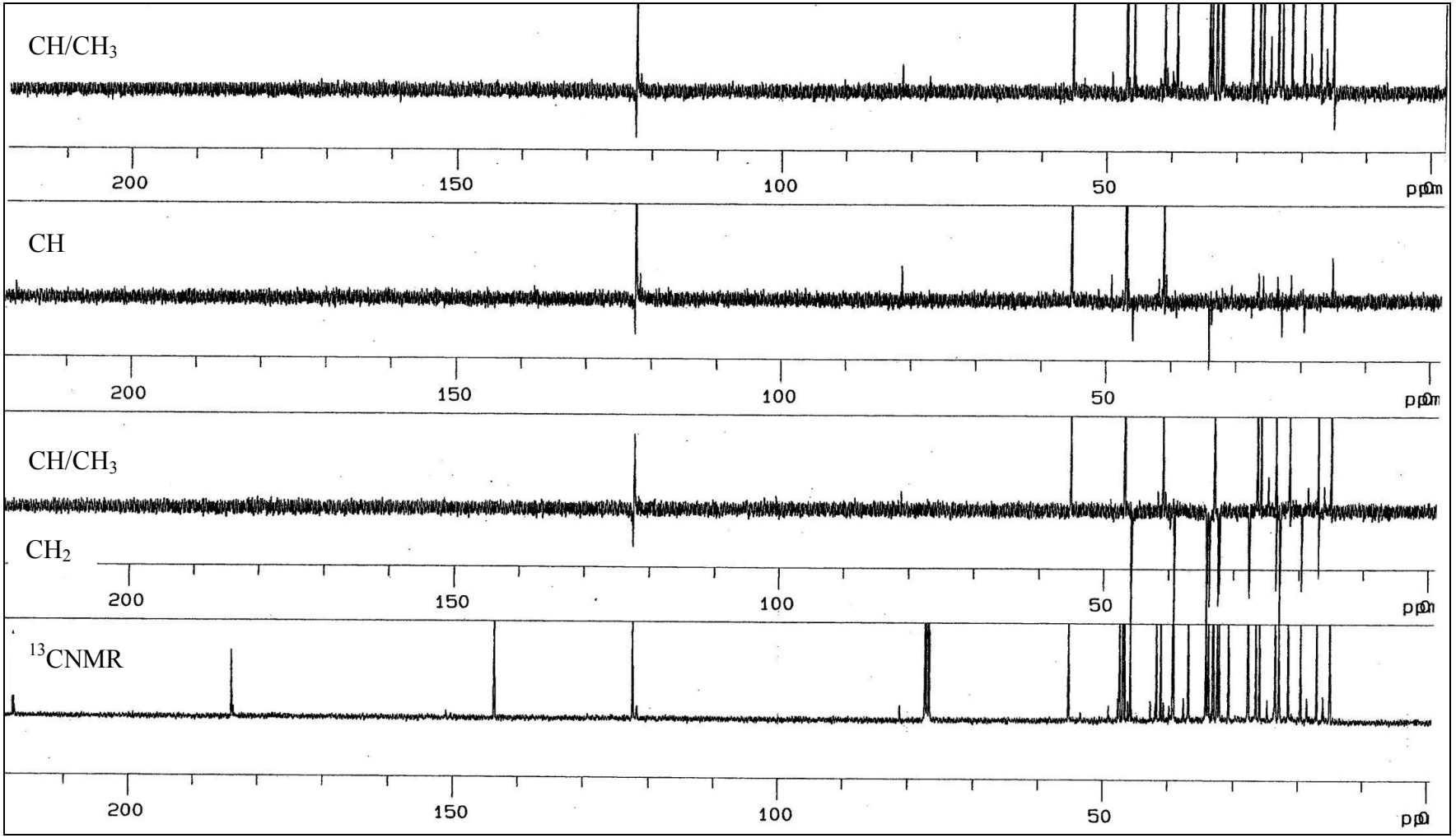


Figure 3.4.3: DEPT Spectrum of Compound D

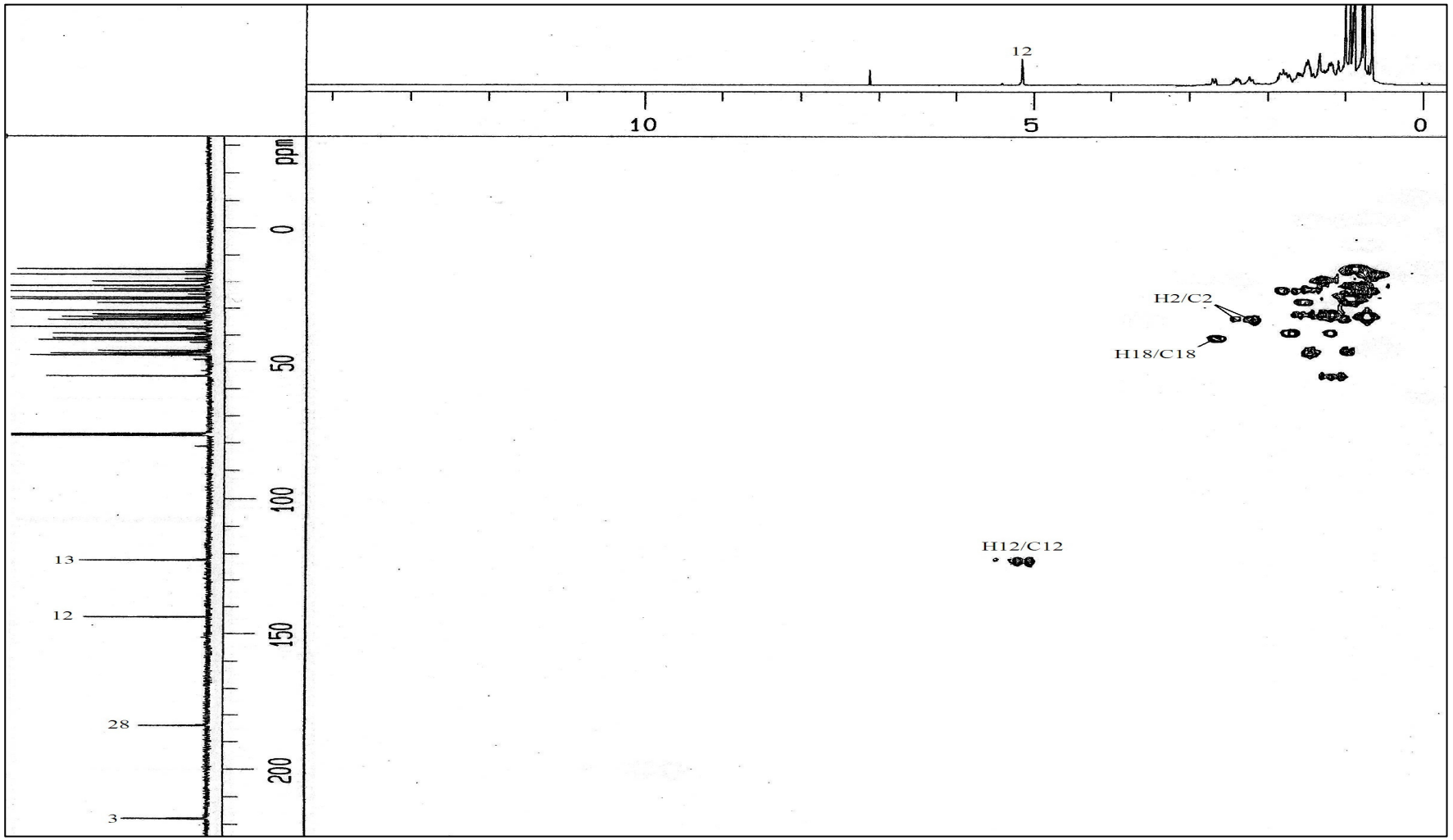


Figure 3.4.4: HSQC Spectrum of Compound D

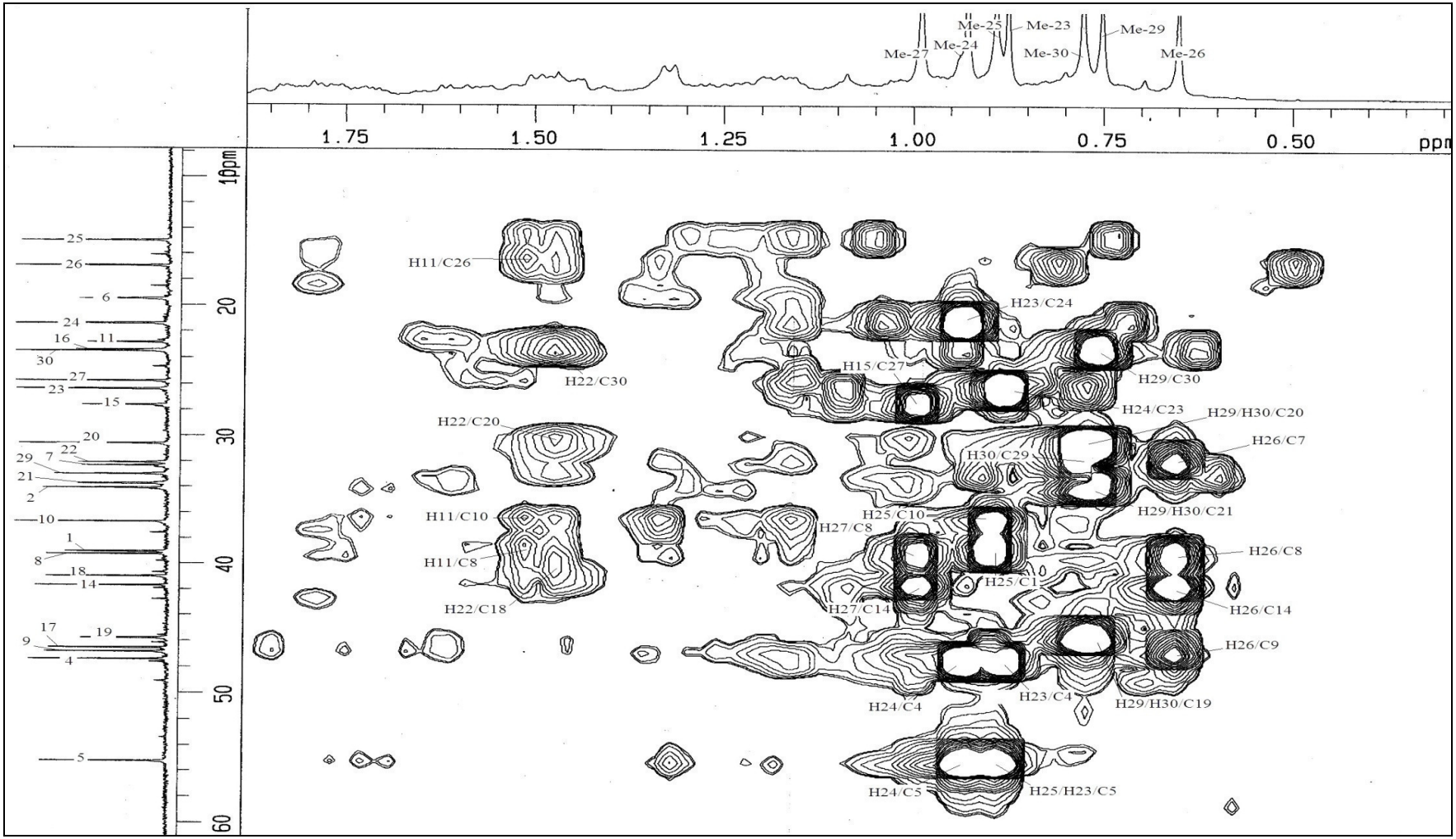
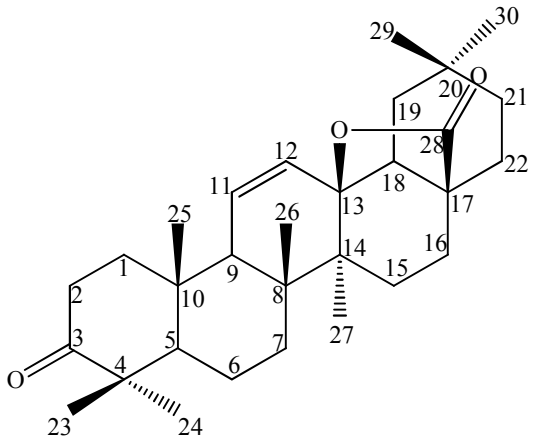


Figure 3.4.5: HMBC Spectrum of Compound D

3.5 Compound E : 3-oxoolean-11-en-13β(28)-olide 119



Compound E was obtained as gummy oil and had a molecular formula of $C_{30}H_{44}O_3$, established by the EIMS spectrum (M^+, *m/z* 452). The IR spectrum of compound E showed an intense bands at 1765, 1705 and 1640 cm^{-1} assigned to a γ-lactone, a six membered ring ketone and a double bond groups respectively.

The ^1H NMR spectrum (Figure 3.5.1) data of compound E were similar to those of compound D. In addition, there were other significant peaks at δ_H5.45 (*dd*, *J* = 10.3, 3.2 Hz, H-11)) and δ_H6.03ppm (*dd*, *J* = 10.3, 1.3 Hz, H-12) suggesting the presence of a 13β, 28 olide moiety[51,52].

The ^{13}C NMR spectrum (Figure 3.5.2) of compound E exhibited signals at δ_C 216.9(C-3), 135.3(C-11), 127.4(C-12), 89.6(C-13) and 180.0(C-28). This spectral feature indicate that compound E belongs to an oleanan-28,13β-olides system.

The C<u>H</u>$_3$-23 (δ_H1.08) and the C<u>H</u>$_3$-24 (δ_H1.03) had distinct HMBC (Figure 3.5.3) correlations with the sole carbonyl signal δ_C216.9(C-3) signal. In the HMBC experiment as summarized in Figure 3.5a, the methylene, C<u>H</u>$_2$-15 (δ_H1.20) signals showed cross peaks to the oxygen-bearing quaternary carbon signal at δ_C89.6(C-13), which in turn correlated to

the olefinic proton signal at δ_H6.03 (H-12). Thereby, the disubstituted double bond must be placed at the $\Delta^{11,12}$ position on the C-ring of the oleanane, and also in consideration of eight degrees of unsaturation, the 6 membered ring should be formed *via* a lactone linkage between C-28 and C-13. Thus, the structure of compound E is assigned as 3-oxoolean-11-en-13β(28)-olide[53].

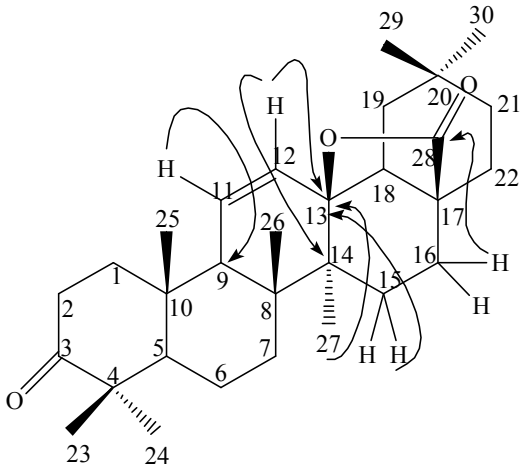


Figure 3.5a : Significant HMBC (→) interaction of Compound E

Table 3.5: ^{1}H, ^{13}C and HMBC Spectral Data of Compound E in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*, Hz)]	HMBC(H→C)
1	39.0	1.34(*m*) 2.10(*m*)	3
2	33.9	2.44(*ddd*, *J*=16.0, 6.8, 3.7Hz) 2.65(*ddd*, *J*=16.0, 11.4, 7.3Hz)	1, 3
3	216.9		
4	47.7		
5	54.7	1.32(*m*)	25
6	18.9	1.58(*br s*)	
7	30.5	1.23(*m*) 1.41(*m*)	6
8	41.5		
9	52.6	1.98(*m*)	8, 10, 11, 12, 25
10	36.1		
11	135.3	5.45(*dd*, *J*=10.3, 3.2Hz)	9, 14
12	127.4	6.03(*dd*, *J*=10.3, 1.3Hz)	13, 14
13	89.6		
14	41.5		
15	25.4	1.20(*m*) 1.72(*m*)	13, 14, 26
16	21.3	1.29(*m*) 2.07(*m*)	17, 28
17	44.0		
18	50.6	2.06(*m*)	
19	37.4	1.34(*m*) 1.79(*m*)	16, 18, 20, 30
20	31.5		
21	34.4	1.29(*m*)	
22	27.1	1.63(*m*)	13
23	26.0	1.08(*s*)	4, 5, 24
24	20.9	1.03(*s*)	4, 5, 23
25	17.3	1.04(*s*)	10,
26	18.2	1.03(*s*)	
27	18.7	1.08(*s*)	7, 8, 13
28	180.0		
29	33.3	0.96(*s*)	19, 20, 21, 30
30	23.6	0.79(*s*)	19, 21, 29,

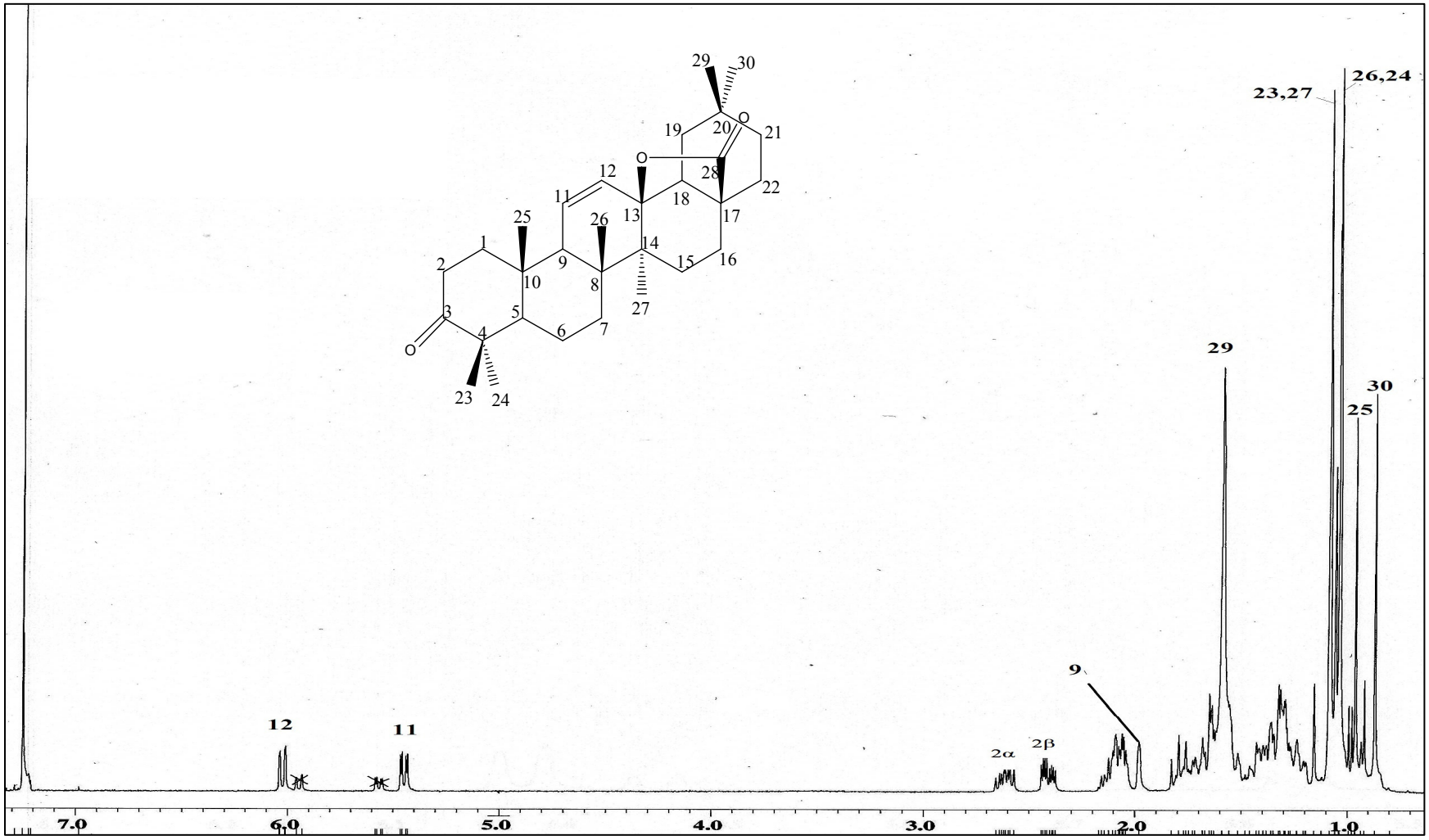


Figure 3.5.1: [1]HNMR Spectrum of Compound E

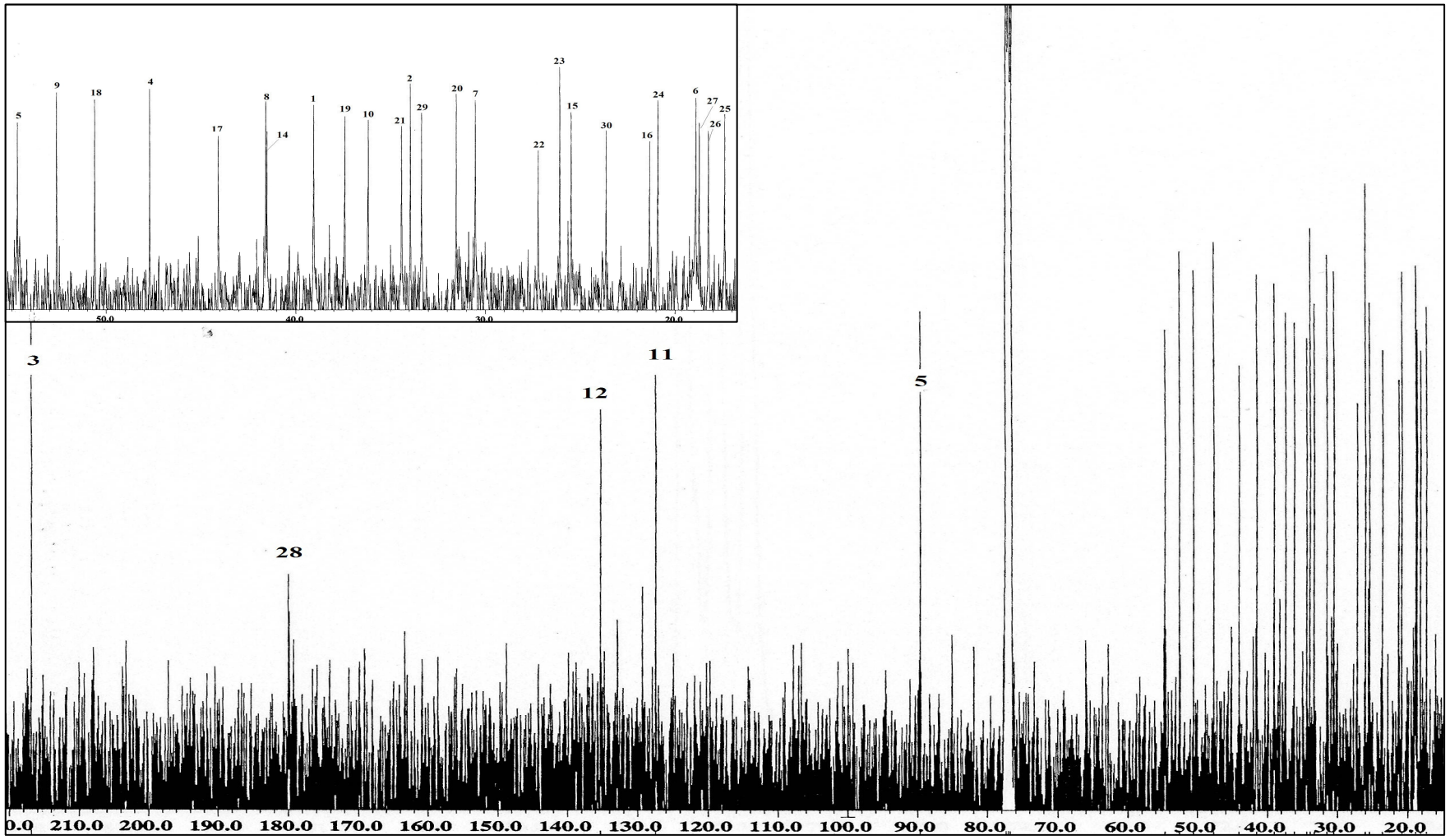


Figure 3.5.2: ^{13}CNMR Spectrum of Compound E

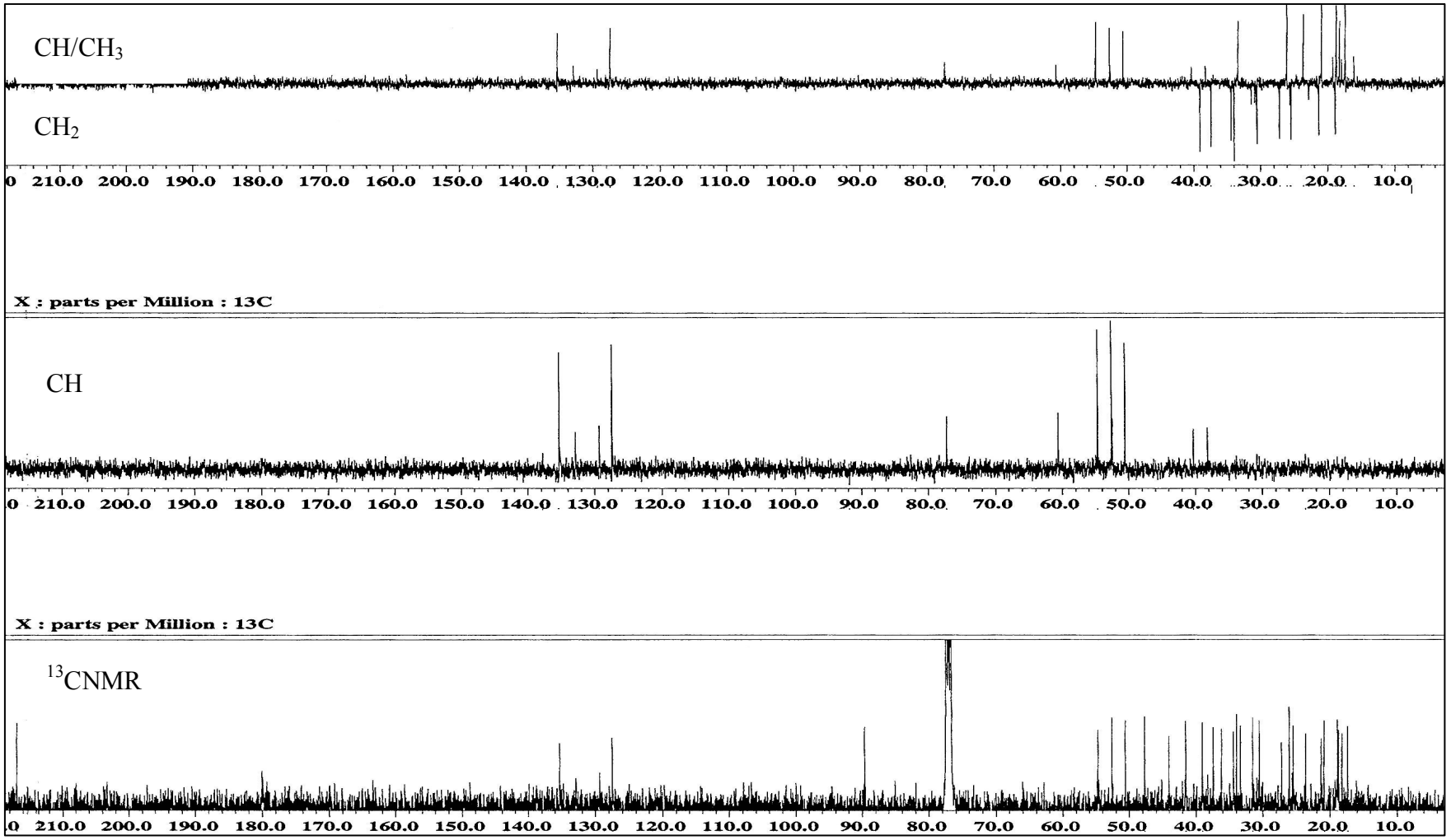


Figure 3.5.3: DEPT Spectrum of Compound E

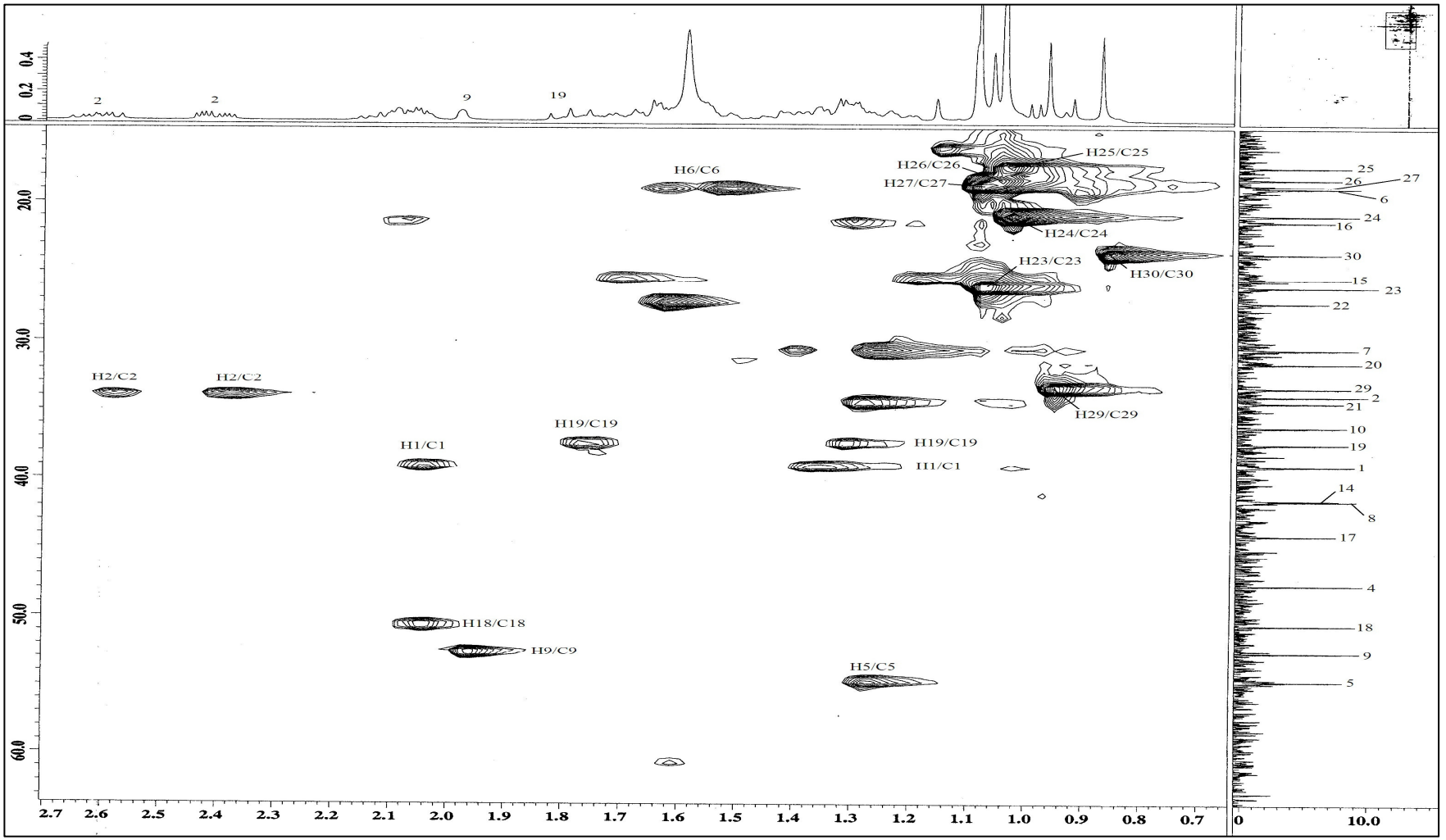


Figure 3.5.4: HSQC Spectrum of Compound E

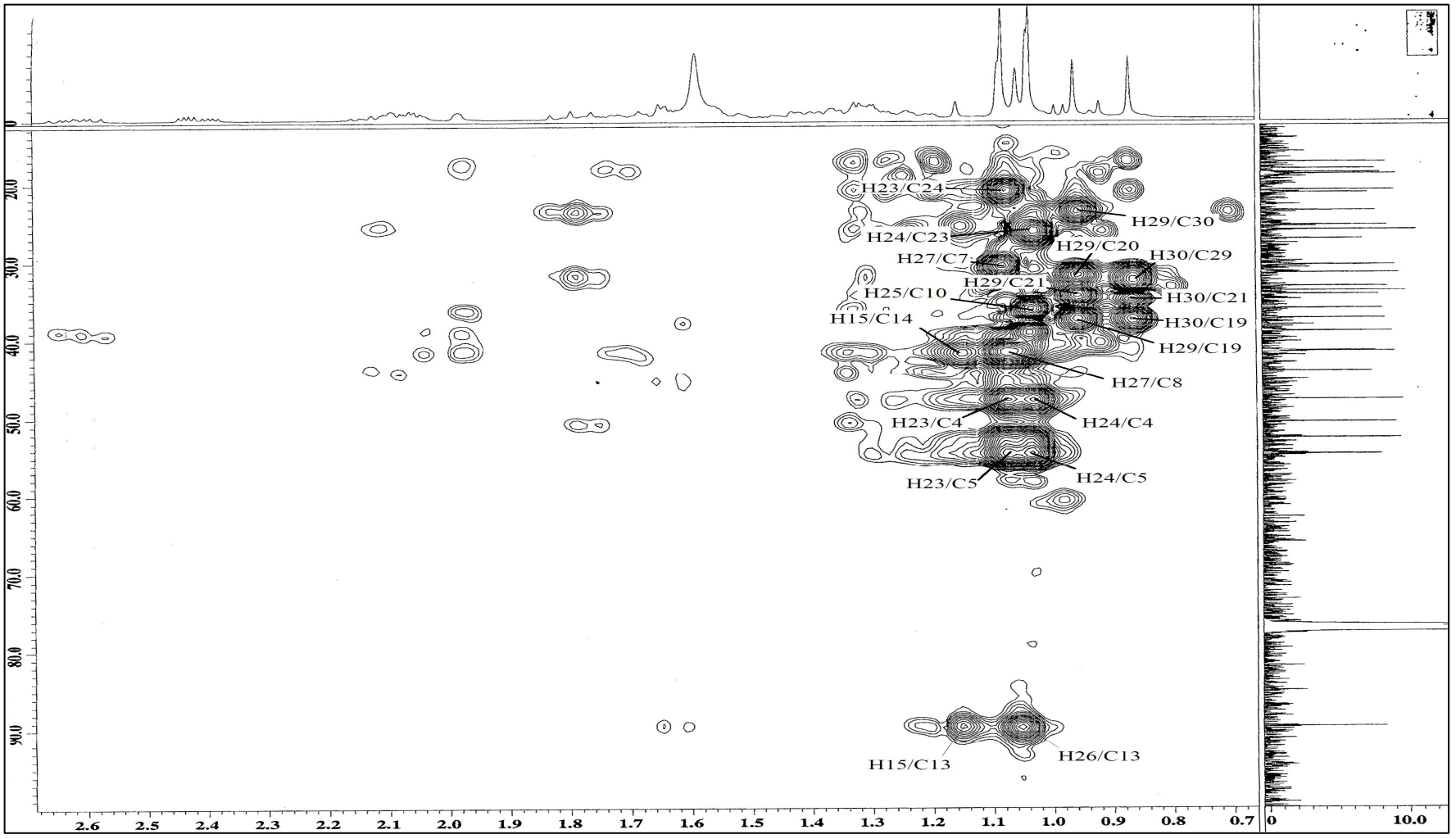
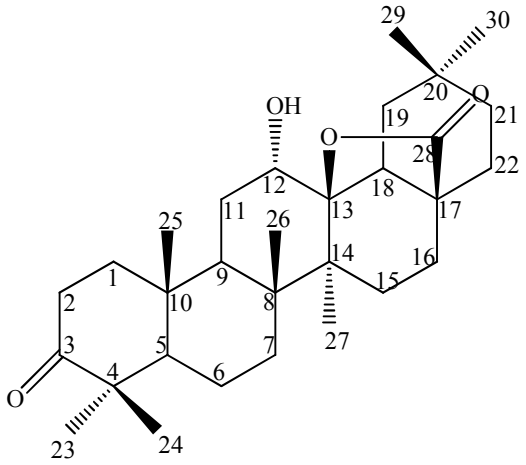


Figure 3.5.5: HMBC Spectrum of Compound E

3.6 Compound F : 12α-hydroxy-3-oxooleanano-28,13-lactone 120



Compound F was isolated as a gummy solid. Its molecular formula $C_{30}H_{46}O_4$ was determined by EIMS *m/z* 470 (M^+). The IR spectrum of compound F displayed absorption at 3619, 1760, 1699 cm^{-1} assigned to hydroxyl group, γ lactone, and a six membered ring ketone respectively.

The ^1H NMR spectrum (Figure: 3.6.1) and ^{13}C NMR spectrum (Figure: 3.6.2) spectroscopic data of compound F were similar to those of compound E except for the presence of a hydroxyl group in compound F instead of the double bond group found in compound E. Moreover, the signal at δ_H3.88 (H-12) attributed to the CH-OH group in the structure of compound F[50]

Furthermore, the ^{13}C NMR spectrum (Figure: 3.6.2) displayed resonances at δ_C 217.7 , 76.3, 90.6 , and 179.8 which were assigned to C-3, C-12, C-13 and C-28 respectively which suggesting the presence of a 13β, 28 olide and 12-hydroxyl group.

This was further corroborated by the HMBC spectrum (Figure: 3.6.3), whereby the proton signal at δ_H3.88(H-12) correlated with C-13 (δ_C90.6) and C-18 (δ_C43.9) confirmed the 12α-hydroxy-3-oxooleanano-28,13-lactone structure[54] of compound F. The below assignments (Figure 3.6a) were made by 2D-NMR studies including HMBC (Table 3.6)

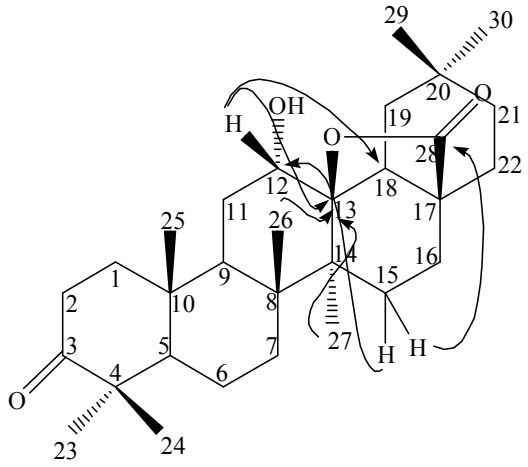


Figure 3.6a : Significant HMBC (→) interaction of Compound F

Table 3.6: ^{1}H, ^{13}C and HMBC Spectral Data of Compound F in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*,Hz)]	HMBC(H→C)
1	39.6	1.87(*m*)	25
2	34.2	2.4-2.5(*m*)	1, 3
3	217.7		
4	47.4		
5	54.4	1.34(*m*)	
6	19.1	1.46(*m*)	25
7	33.4	1.25(*m*)	
8	42.2		
9	51.2	1.99(*m*)	4, 5, 8
10	36.2		
11	21.2	2.11(*m*)	18
12	76.3	3.88(*s*)	13, 18
13	90.6		
14	42.2		
15	29.1	2.01(*m*) 1.45(*m*)	9, 12, 14, 17, 28
16	27.5	1.59(*m*)	
17	47.4		
18	43.9	1.70(*m*)	14, 15, 19, 27
19	39.5	1.42(*m*)	14, 20, 22
20	31.6		
21	34.0	1.28(*m*)	
22	28.1	1.14(*m*)	17, 18, 20, 21
23	26.7	1.07(*s*)	3, 4, 5, 24
24	21.1	1.02(*s*)	3, 4, 5, 23
25	16.3	0.96(*s*)	5
26	18.2	1.16(*s*)	7, 13, 14
27	18.5	1.29(*s*)	8, 9, 13, 15
28	179.8		
29	33.3	0.96(*s*)	18, 19, 20, 21
30	23.9	0.88(*s*)	19, 20, 21, 29

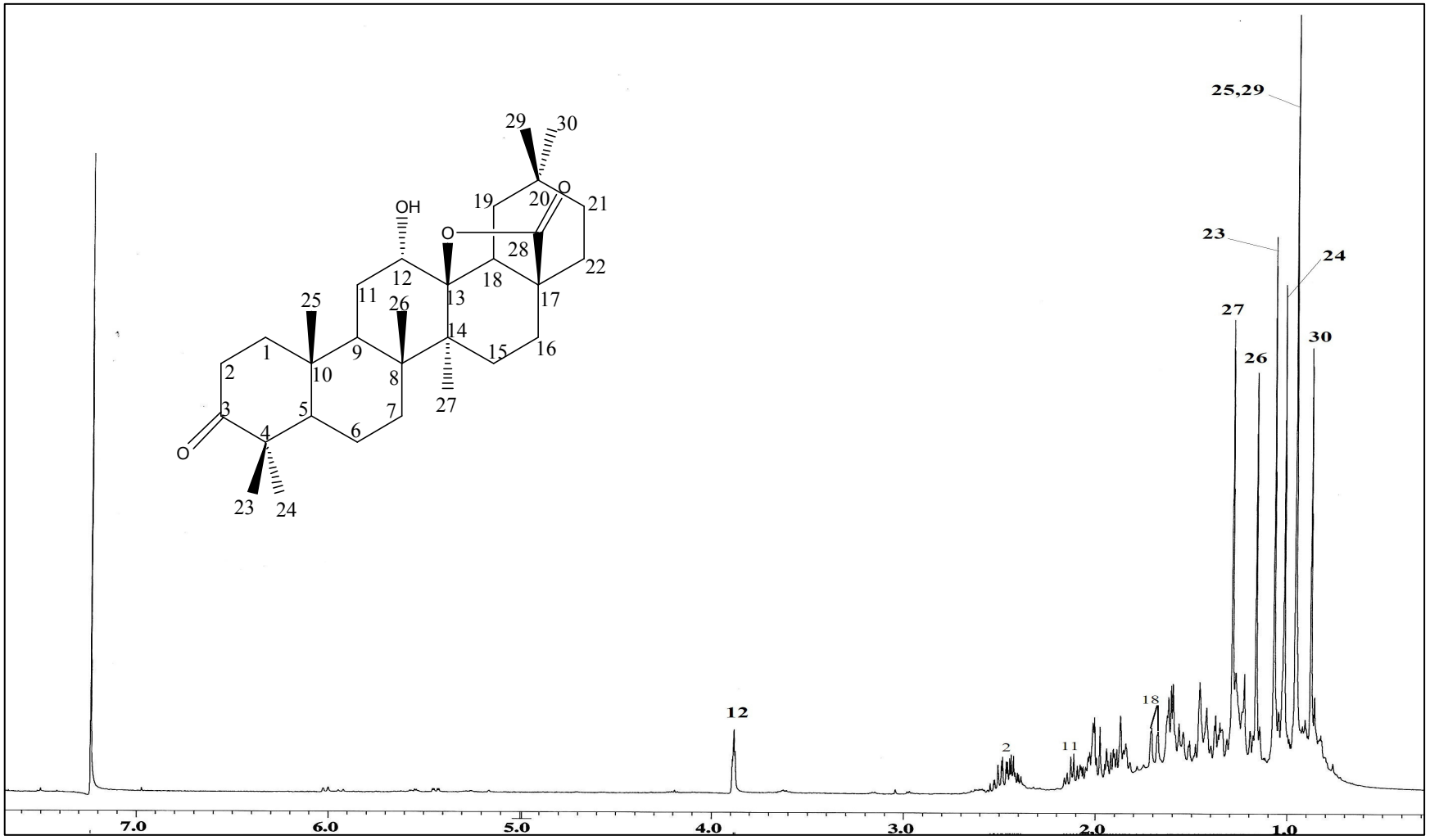


Figure 3.6.1: [1]HNMR Spectrum of Compound F

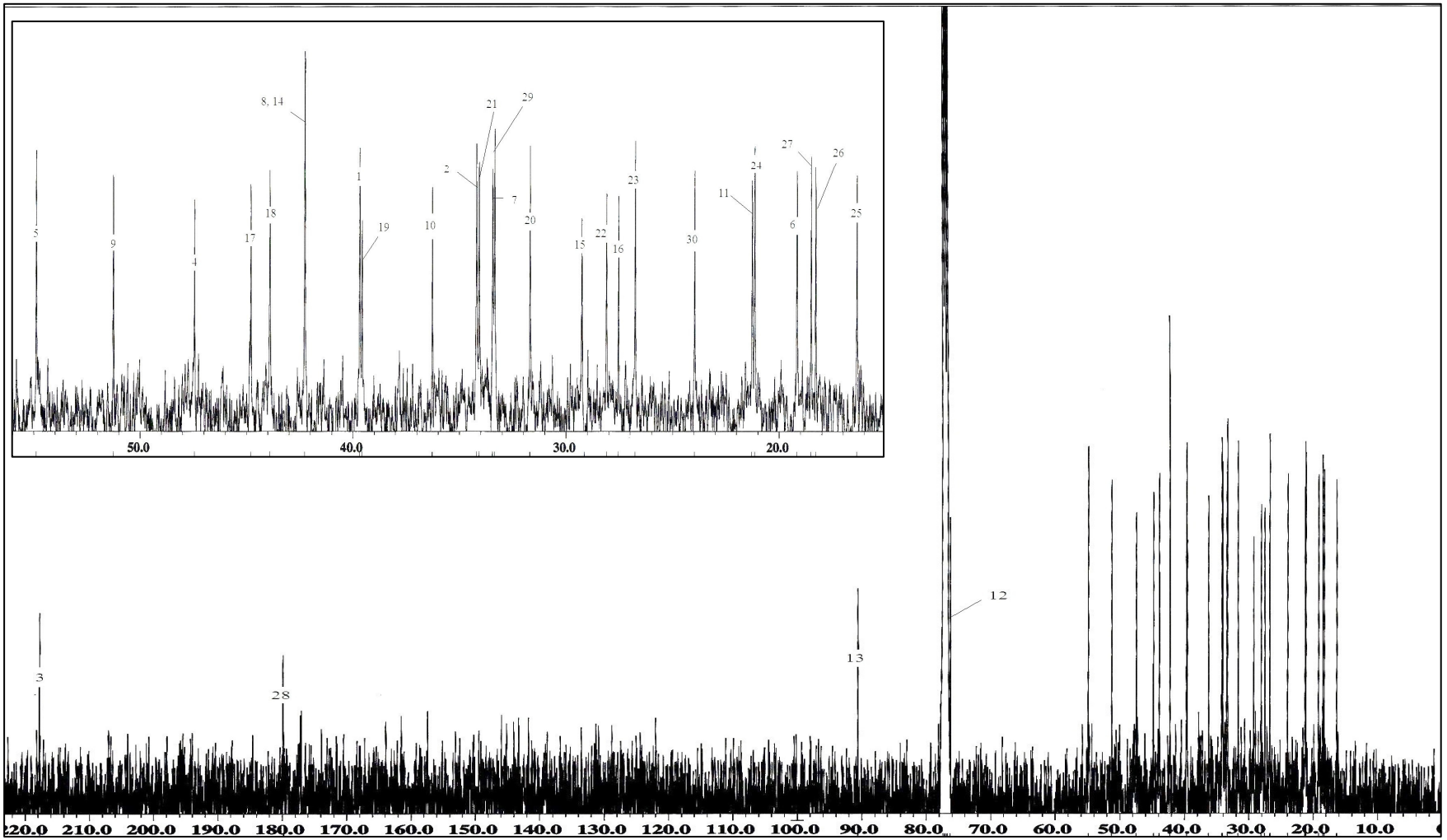


Figure 3.6.2: ^{13}CNMR Spectrum of Compound F

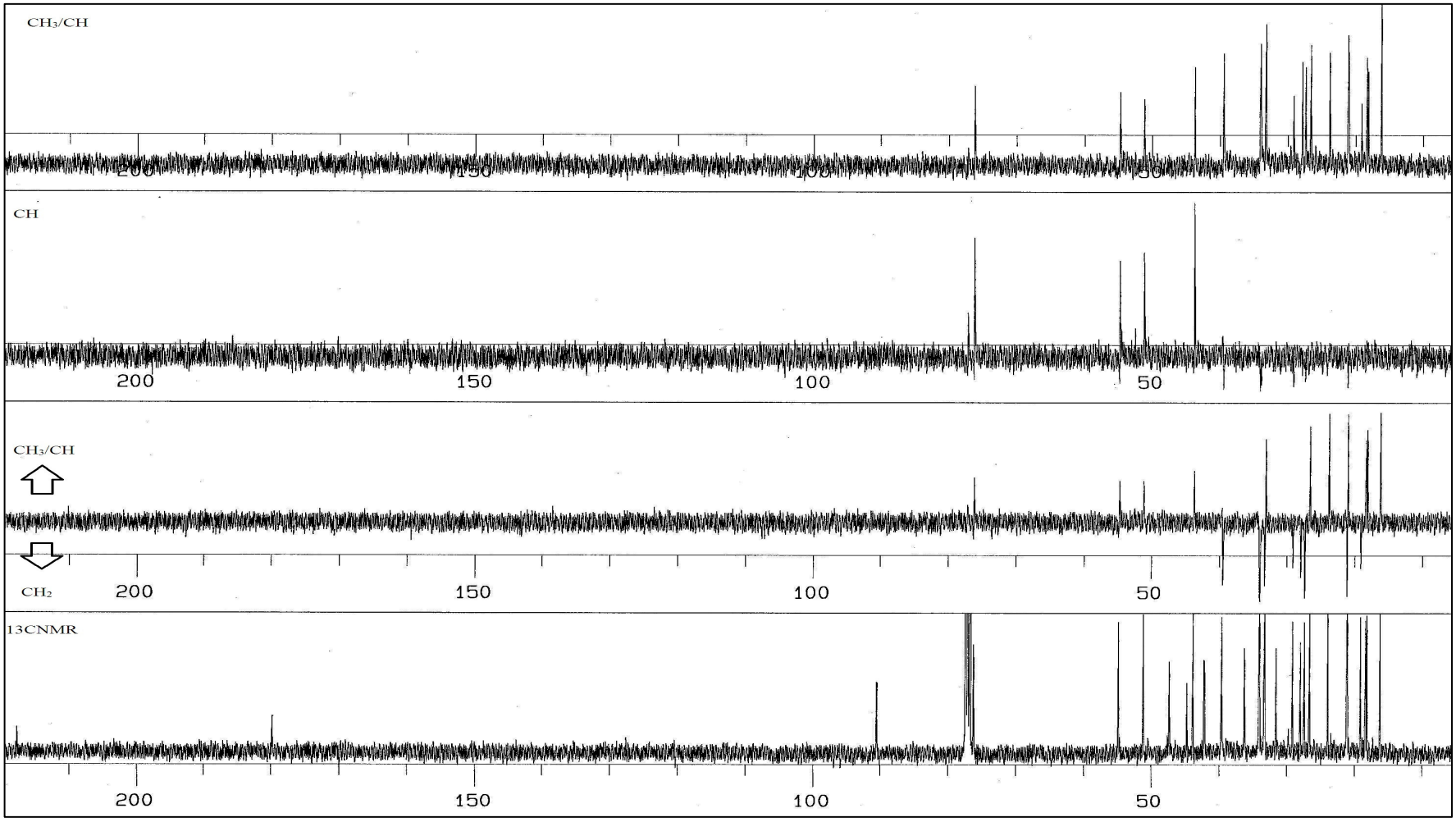


Figure 3.6.3: DEPT Spectrum of Compound F

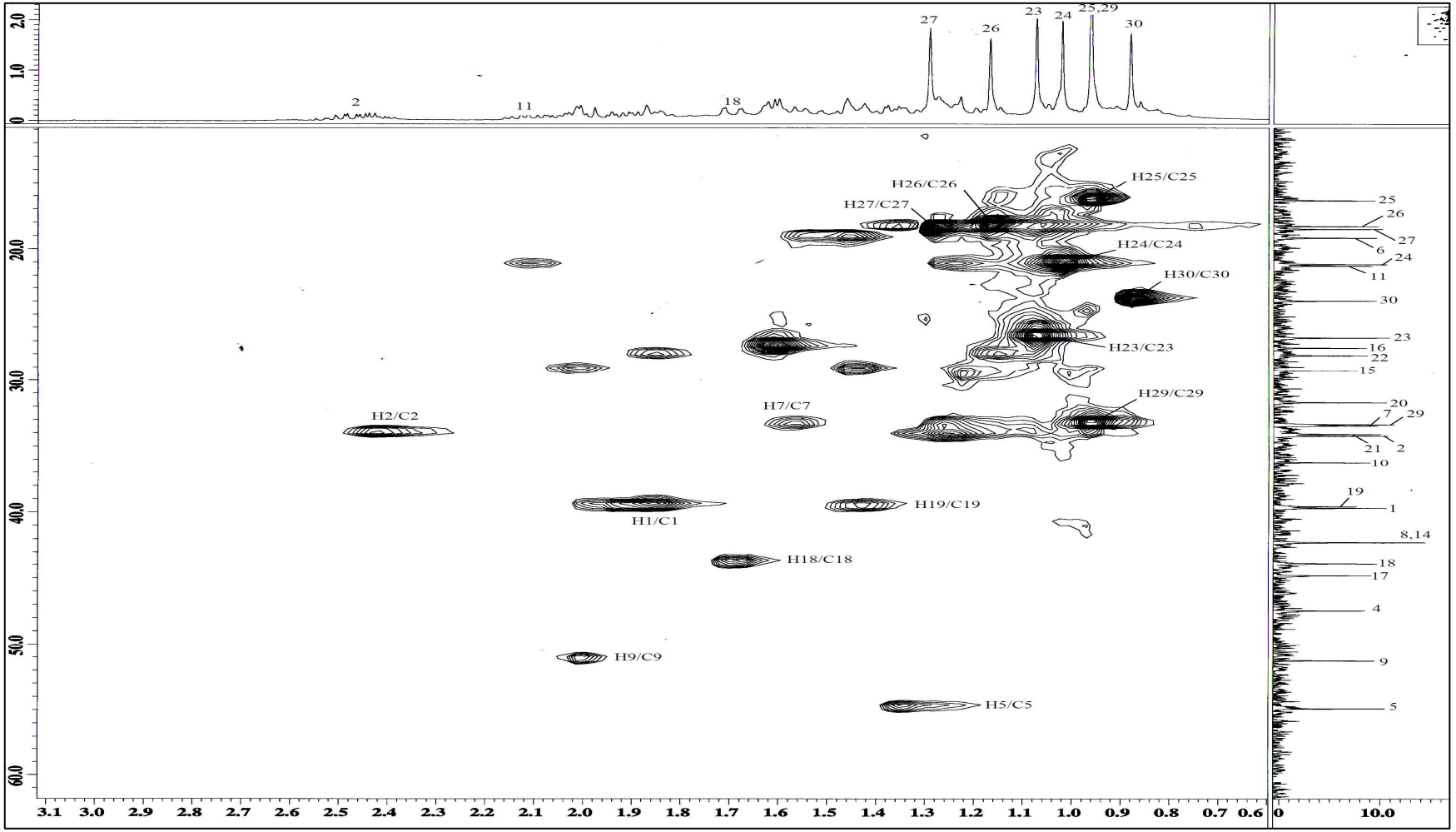


Figure 3.6.4: HSQC Spectrum of Compound F

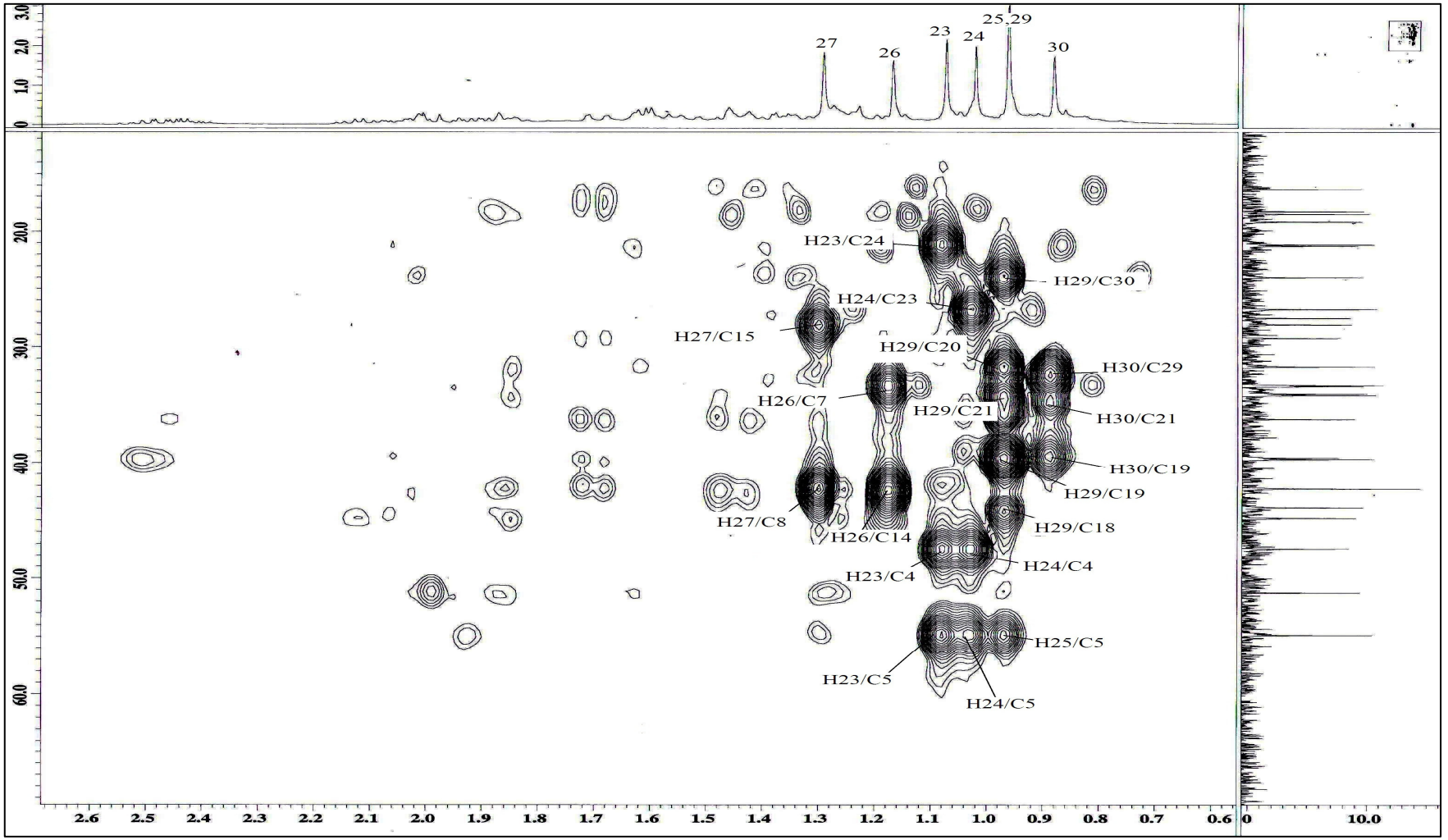
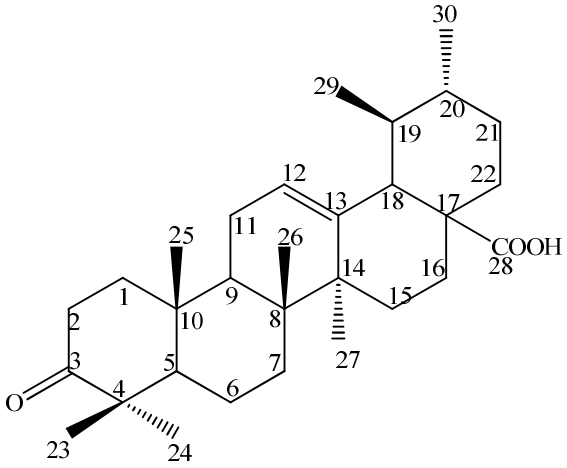


Figure 3.6.5: HMBC Spectrum of Compound F

3.7 Compound G : 3-oxoursolic acid 121



Compound G was obtained as a white amorphous powder. Its was considered to be a constitutional isomer of compound D on the basis of EIMS data which showed *m/z*: 454.37 $(M)^+$, ^1H NMR and ^{13}C NMR (Table 3.7) together with the fact that the molecular formula, $C_{30}H_{47}O_3$, consistent with eight degrees of unsaturation. Its IR spectrum showed absorption bands at 3400-2920, 1695 and 1670 cm^{-1} assigned to a hydroxyl acid, a six membered ring ketone and a double bond groups respectively.

In ^1H NMR spectrum (Figure 3.7.1) of Compound G was deduced to be an ursane due to five singlet methyl groups at δ_H1.06;C$\underline{H}_3$-23, 1.06;C$\underline{H}_3$-25, 1.02;C$\underline{H}_3$-27, 1.00;C$\underline{H}_3$-24 and 0.80;C$\underline{H}_3$-26 and two as doublets at δ_H0.84 (*J*=6.3 Hz;C$\underline{H}_3$-29) and 0.93 (*J*=7.8Hz;C$\underline{H}_3$-30)[55]. It also showed resonance for an olefinic proton at δ_H5.23 of H-12 and a characteristic methine proton doublet at δ_H2.16 (*J*=11.4Hz, H-18)[49].

In addition , the ^{13}C NMR spectrum (Figure: 3.7.2) of compound G revealed the presence of seven methyls, nine methylenes, six methine and eight quaternary carbons which provided further evidence for a pentacyclic triterpene skeleton. The ^{13}C NMR spectrum of

Compound G displayed resonances at δ_C125.6, 138.1, 184.0 and 217.9 attributable to C-12, C-13, C-28 and C-3 respectively.

The HMBC spectrum (Figure :3.7.3),showed correlation between δ_C125.6(C-12) and δ_C138.1(C-13) with δ_H2.16[*d*, *J*=11.4Hz;(H-18)]. Thorough analysis of 1D and 2D spectra of compound G confirmed that it is the known as 3-oxoursolic[55]. The below assignments (Figure 3.7a) were made by 2D-NMR (Table 3.7) studies including HMBC (Figure : 3.7.3)

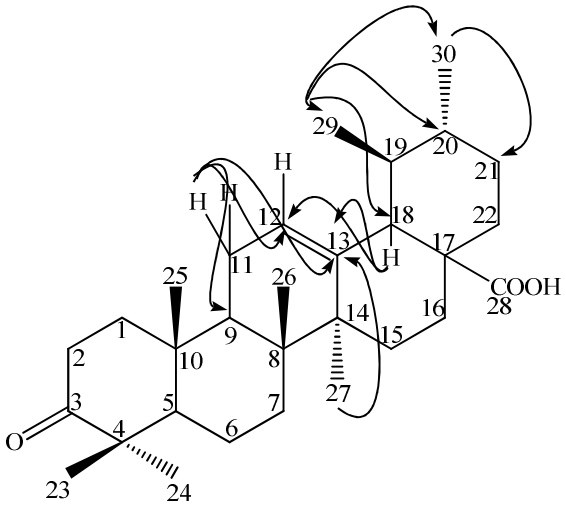


Figure 3.7a : Significant HMBC (→) interaction of Compound G

Table 3.7: ^{1}H, ^{13}C and HMBC Spectral Data of Compound G in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*, Hz)]	HMBC(H→C)
1	39.3	1.27(*m*)	3
		1.37(*m*)	
2	34.2	2.52(*m*)	1, 3
		2.36(*m*)	
3	217.9		
4	47.4		
5	55.3	1.30(*m*)	6, 7, 23, 24, 25,
6	19.6	1.45(*m*)	5
7	32.5	1.33(*m*)	
		1.40(*m*)	
8	39.5		
9	46.8	1.58(*m*)	1, 11, 14, 26
10	36.7		
11	23.4	1.96(*m*)	
		1.99(*m*)	
12	125.6	5.23(*s*)	11
13	138.1		11
14	42.1		
15	28.0	1.11(*m*)	
16	24.1	1.60(*m*)	22
		1.68(*m*)	
17	48.0		
18	52.6	2.16(*d, J*=11.4Hz)	12, 13, 14, 16, 19, 28, 29
19	39.1	1.85(*m*)	16
20	38.8	0.88(*m*)	29,
21	30.6	1.50(*m*)	22
22	36.7	1.63(*m*)	
		1.72(*m*)	
23	26.6	1.06(*s*)	3, 4, 5, 24
24	21.5	1.00(*s*)	3, 4, 5, 23
25	15.2	1.06(*s*)	1, 10
26	17.0	0.80(*s*)	7, 8, 9, 14
27	23.5	1.02(*s*)	8, 13, 14, 15
28	184.0		
29	17.0	0.84(*d*, *J*=6.3Hz)	18, 20, 30
30	21.2	0.93(*d*, *J*=7.8Hz)	21, 29,

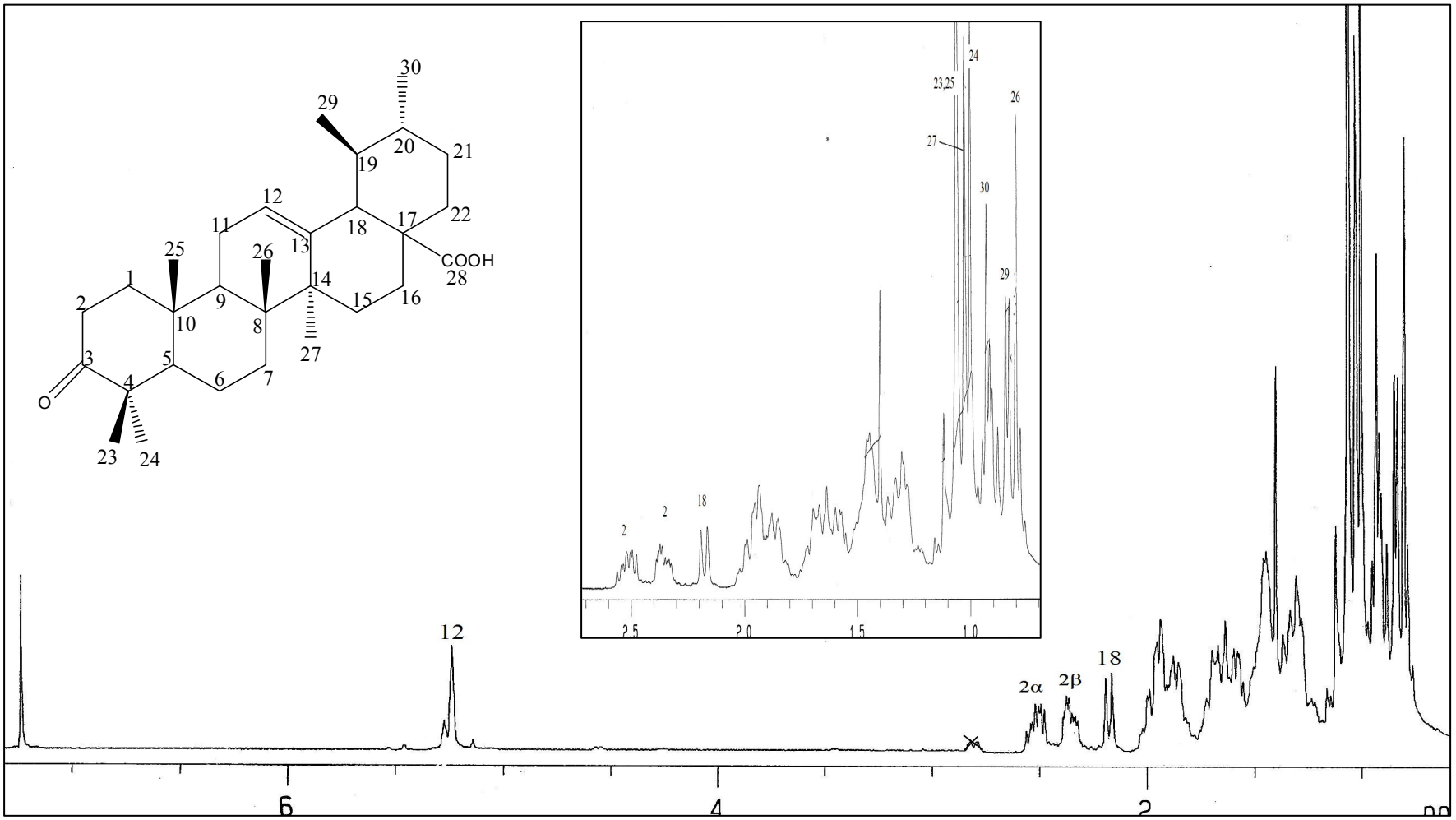


Figure 3.7.1: [1]HNMR Spectrum of Compound G

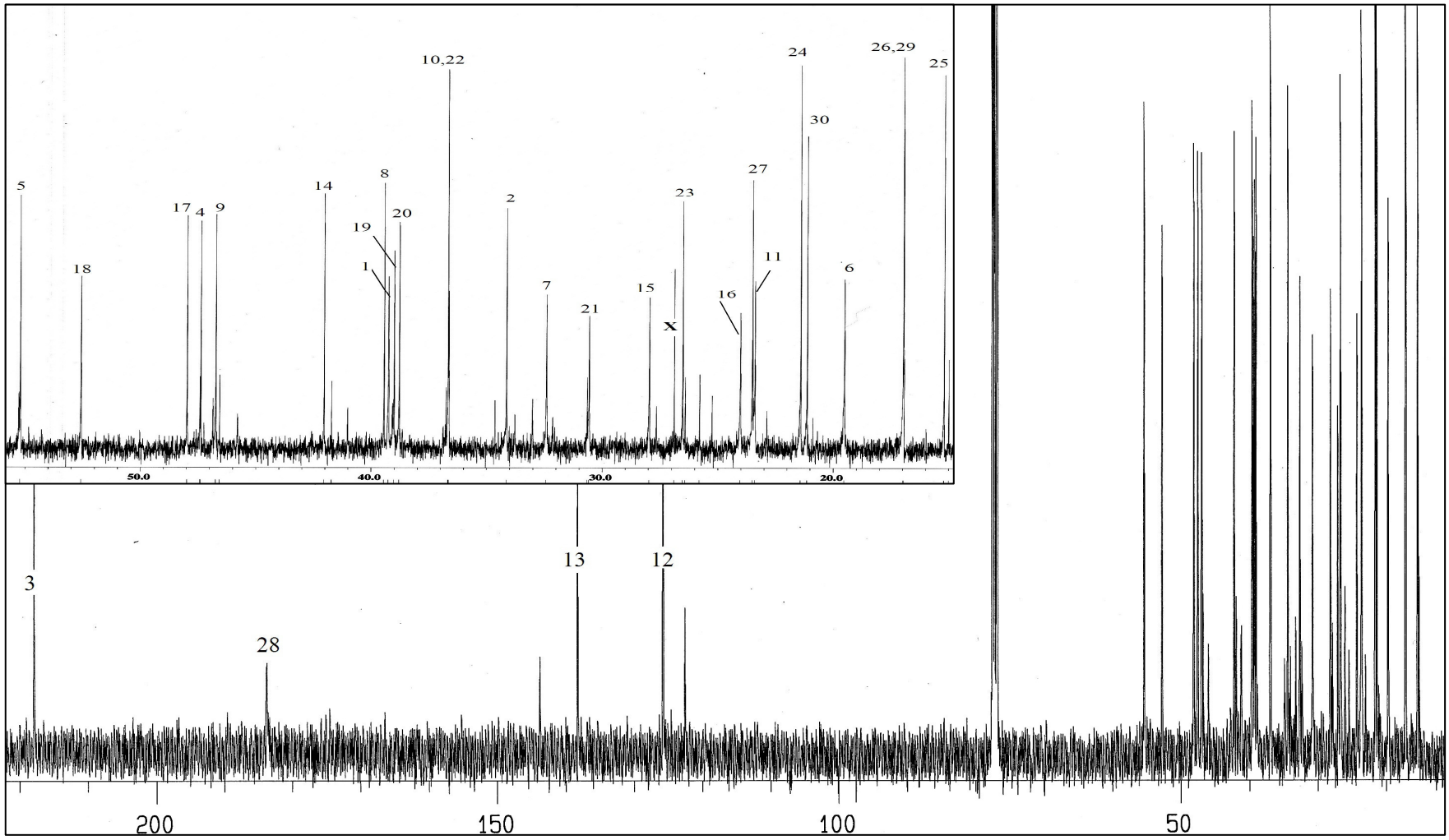


Figure 3.7.2: ^{13}CNMR Spectrum of Compound G

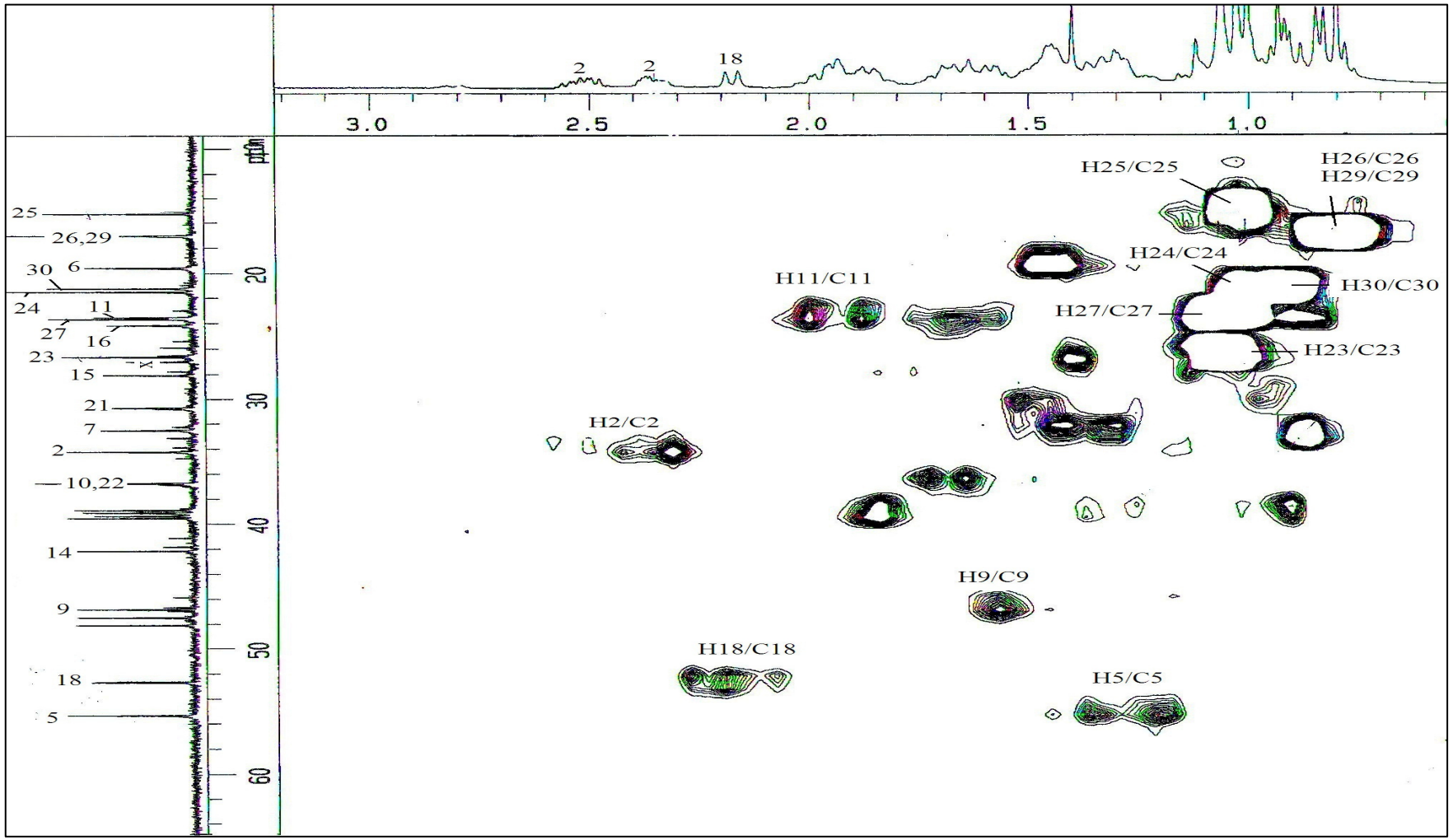


Figure 3.7.3: HSQC Spectrum of Compound G

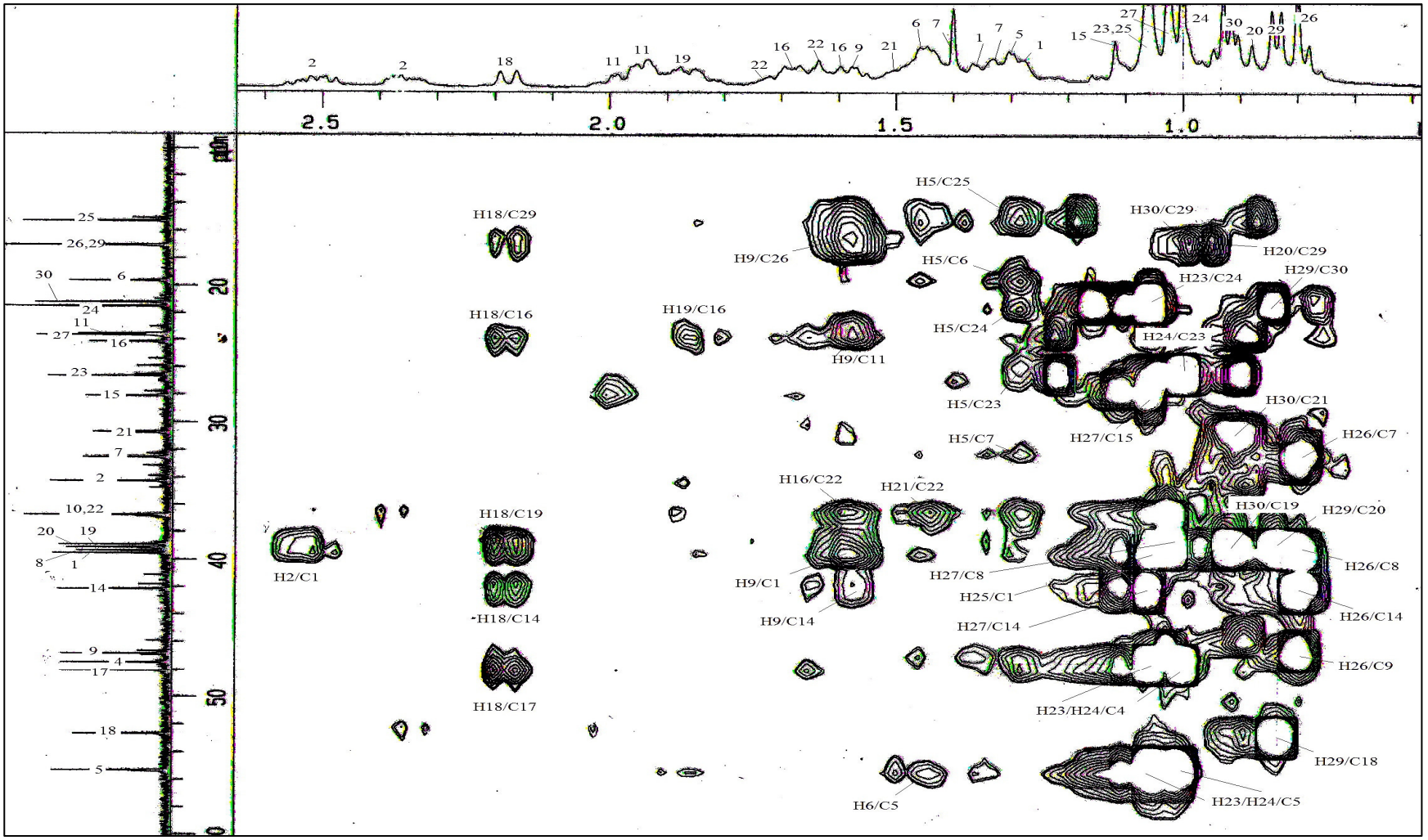
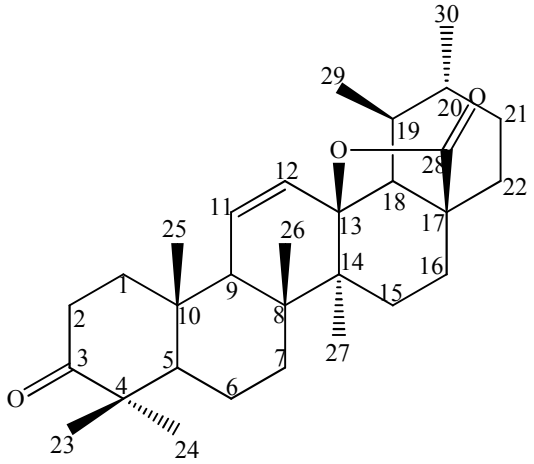


Figure 3.7.4: HMBC Spectrum of Compound G

3.8 Compound H : 3-ketours-11-en-13β(28)-olide 122



Compound H is a constitutional isomer of compound E based on its molecular formula of $C_{30}H_{44}O_3$ (M^+ ,*m/z*:452.33) which was determined from LCMS and ^{13}C NMR (Figure: 3.8.2). Its IR spectrum showed absorption bands at 2634, 2866 (CH), 1765 (lactone C=O), 1703 (ketone C=O), and 1137 cm^{-1} (C-O).

Compound H presented spectral data similar to those of ring A of compound G. Its ^{1}HNMR spectrum (Figure : 3.8.1) showed five tertiary methyl signals observed as singlets δ_H1.03;C$\underline{H}_3$-24, 1.04;C$\underline{H}_3$-25, 1.08;C$\underline{H}_3$-23, 1.08;C$\underline{H}_3$-26, 1.15;C$\underline{H}_3$-27 and two methyls were observed as doublets [δ_H0.99 (*J*=6.4Hz;C$\underline{H}_3$-29) and 0.93 (*J*=6.4Hz;C$\underline{H}_3$-30)]. In the ^{1}H NMR of compound H, 2 vinylic proton signals were observed belonging to H-11 [δ_H5.95 (*J* = 10.5, 1.3Hz)] and H-12 [δ_H5.57 (*J* = 10.5, 3.2Hz)] instead of only one vinylic proton signal in compound G. This is because the double bond in compound H is on C-11 and C-12 but in compound G the double bond is on C-12 and C-13.

The structure was finally elucidated from interpretation of its ^{13}C NMR spectral data (Table 3.8) which showed the presence of the signal at δ_C216.9 attributable to the ketone in position C-3.

Further analysis on the HMBC spectrum also led to the identification of a signal at δ_C89.2 (C-13) which was shown in (Figure: 3.8.3) correspond to an olefinic methine proton at δ_H5.95 (*dd*, *J*=10.5, 1.3). The signal at δ_C180.1 (C-28) was attributed to C-28 because of the presence of the lactonic system in ring-D between C-28 carbonyl function and C-13. On the basis of the above spectra evidence the structure of compound H was established as 3-ketours-11-en-13β(28)-olide[56]. The complete assignments (Figure 3.8a) were accomplished by 2D-NMR studies including HMBC (Table 3.8)

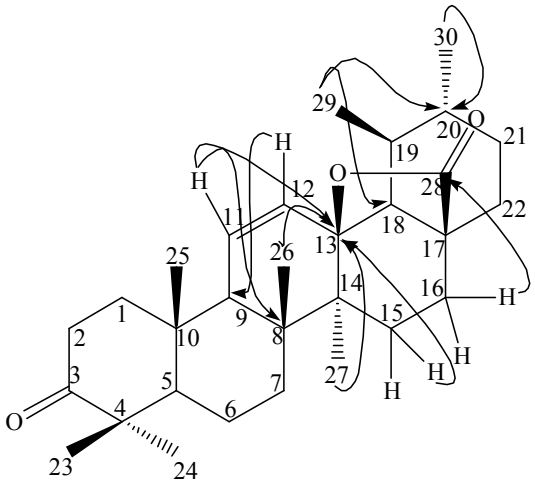


Figure 3.8a : Significant HMBC (→) interaction of Compound H

Table 3.8: ^{1}H, ^{13}C and HMBC Spectral Data of Compound H in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*,Hz)]	HMBC(H→C)
1	39.1	1.42(*m*)	
		2.08(*m*)	
2	34.0	2.42(*m*)	1, 3
		2.60(*m*)	
3	216.9		
4	47.7		
5	54.7	1.32(*m*)	
6	18.9	1.54(*m*)	
7	30.6	1.56(*m*)	
		1.24(*m*)	
8	41.7		
9	52.5	2.04(*m*)	11, 12, 25
10	36.2		
11	132.9	5.95(*dd*, *J*=10.5, 1.3Hz)	8, 13
12	129.4	5.57(*dd*, *J*=10.5, 3.2Hz)	
13	89.3		
14	42.0		
15	25.6	1.20(*m*)	13
		1.73(*m*)	
16	22.8	1.39(*m*)	28
		2.14(*m*)	
17	45.1		
18	60.6	1.64(*d, J*=12.4Hz)	12, 13, 14, 16, 17, 19
19	38.2	1.76(*m*)	
20	40.3	0.86(*m*)	
21	30.9	1.42(*m*)	
22	31.5	1.52(*m*)	
		1.80(*m*)	
23	26.0	1.08(*s*)	3, 4, 5, 24
24	20.9	1.03(*s*)	3, 4, 5, 23
25	17.3	1.04(*s*)	1, 9, 10
26	18.7	1.08(*s*)	7, 9, 13, 14
27	16.1	1.15(*s*)	8, 13, 15
28	180.1		
29	17.9	0.99(*d*, *J*=6.4Hz)	18, 20
30	19.2	0.93(*d*, *J*=6.4Hz)	20

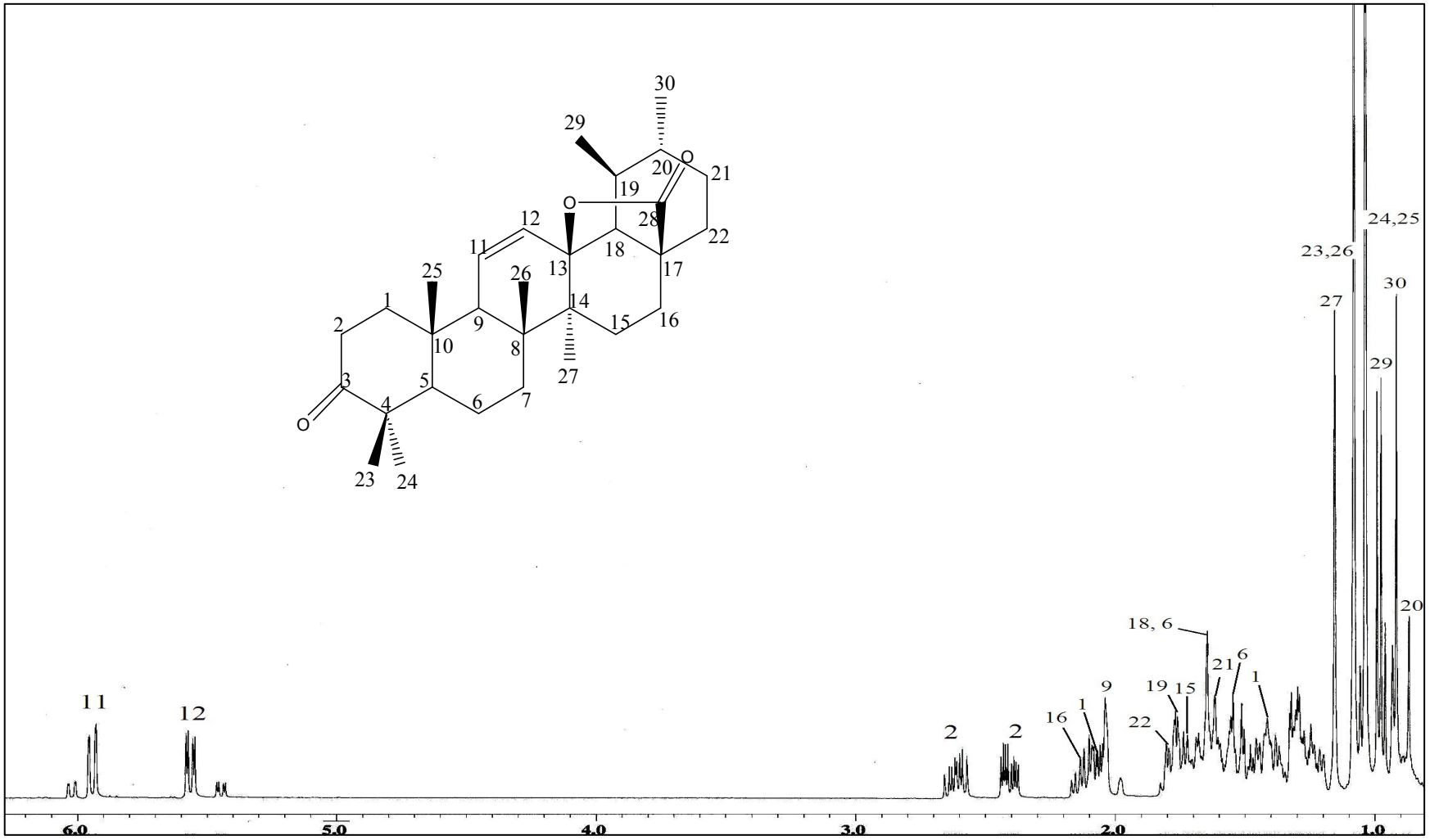


Figure 3.8.1: [1]HNMR Spectrum of Compound H

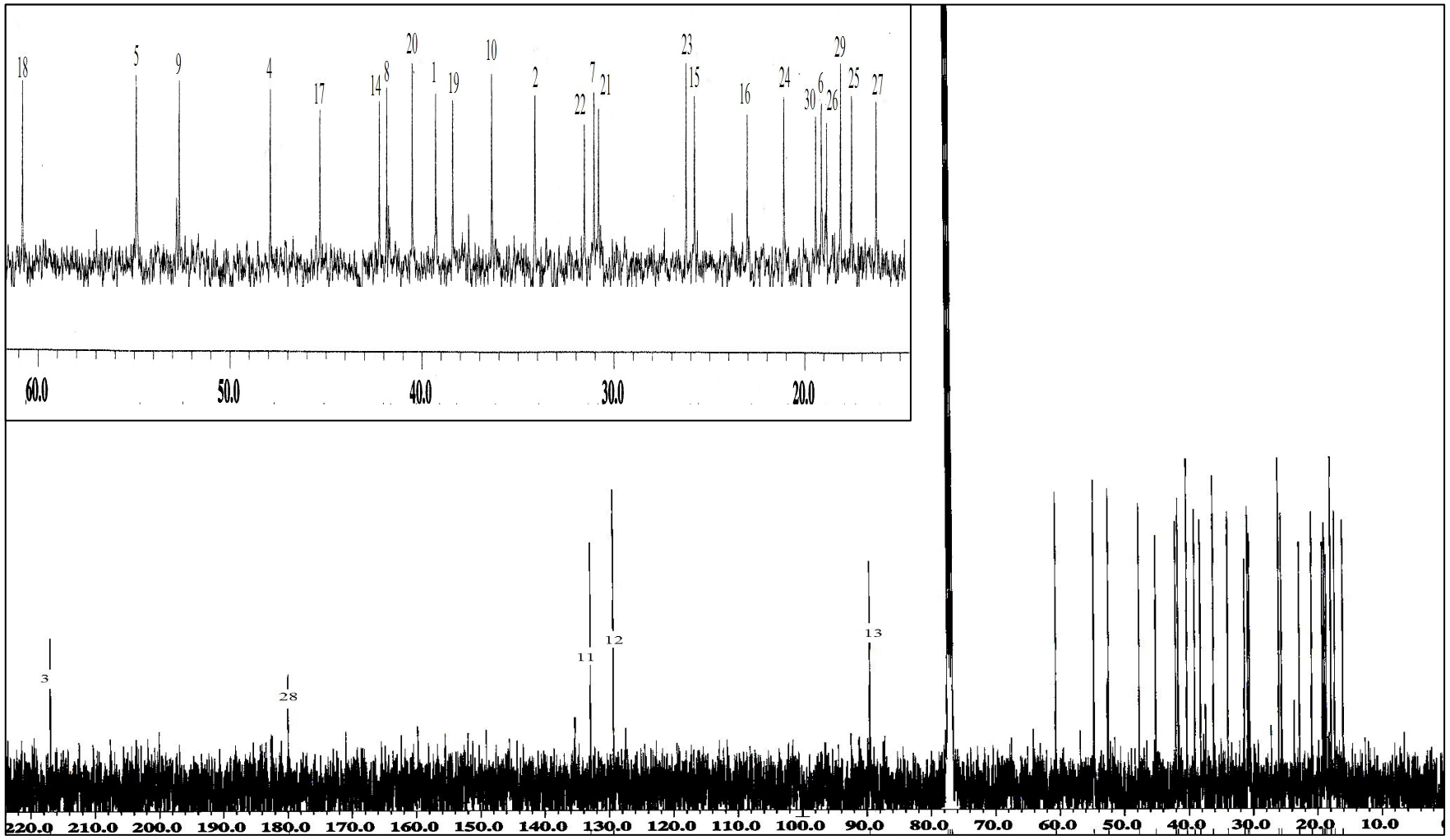


Figure 3.8.2: ^{13}CNMR Spectrum of Compound H

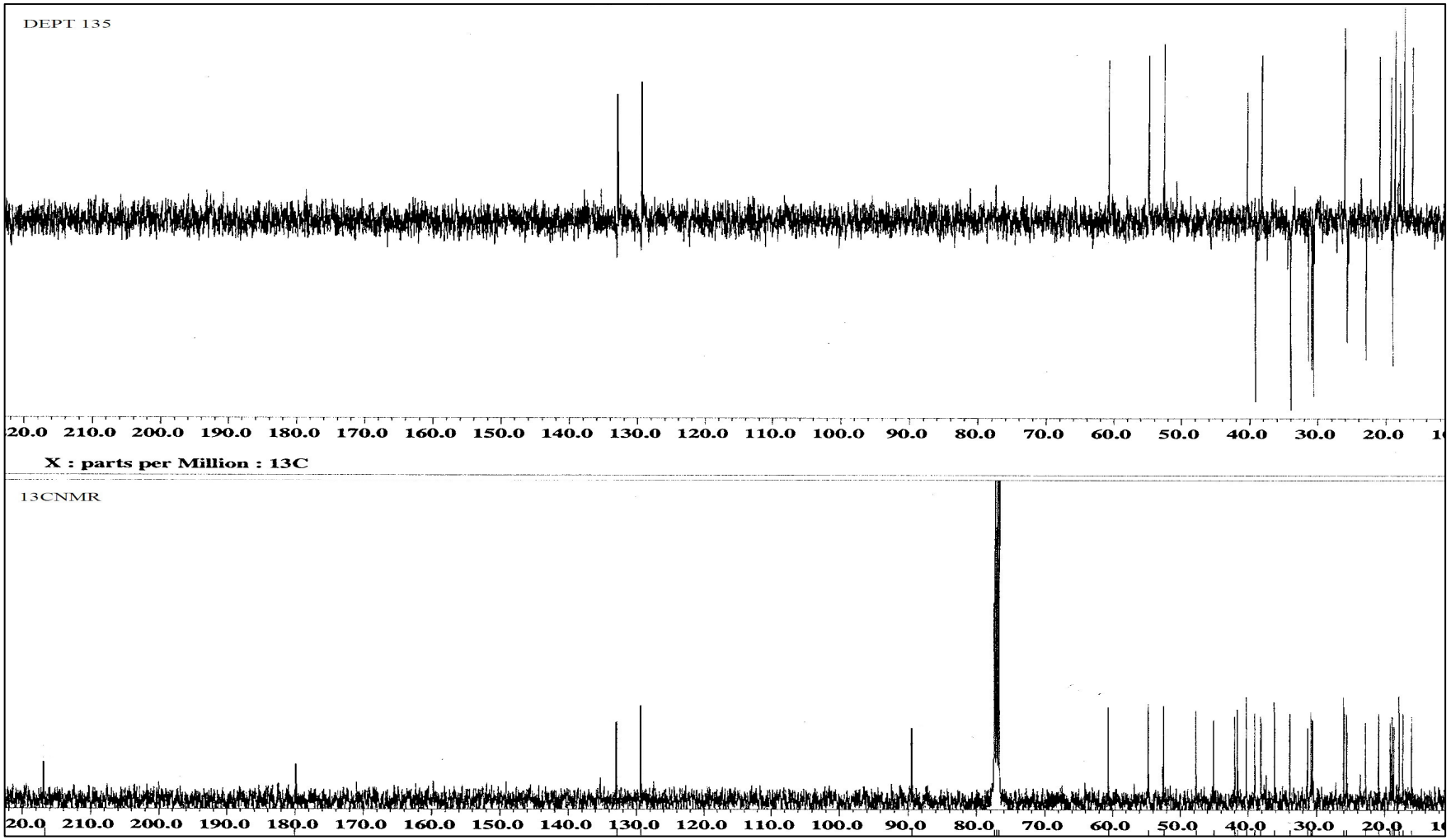


Figure 3.8.3: DEPT Spectrum of Compound H

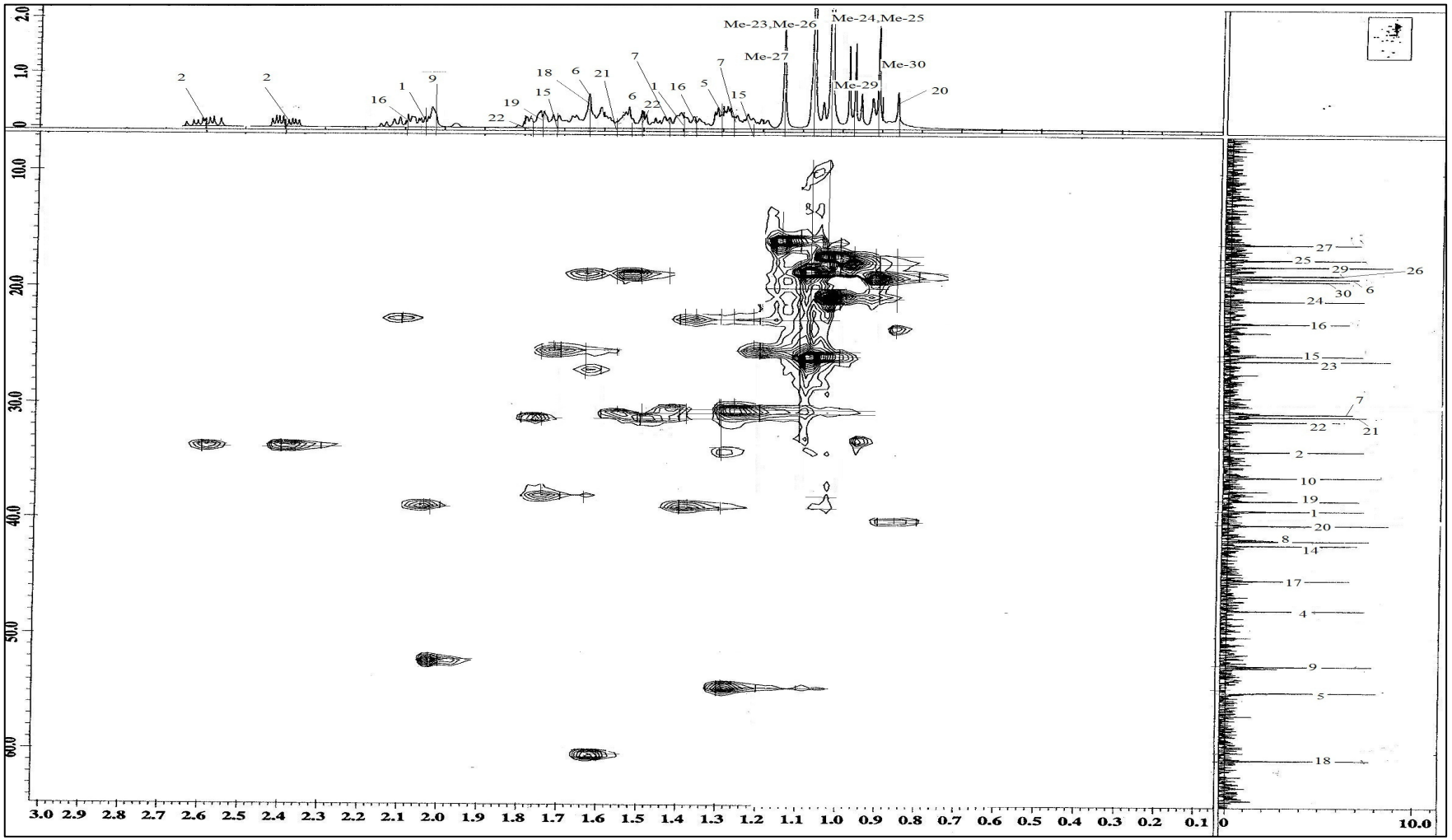


Figure 3.8.4: HSQC Spectrum of Compound H

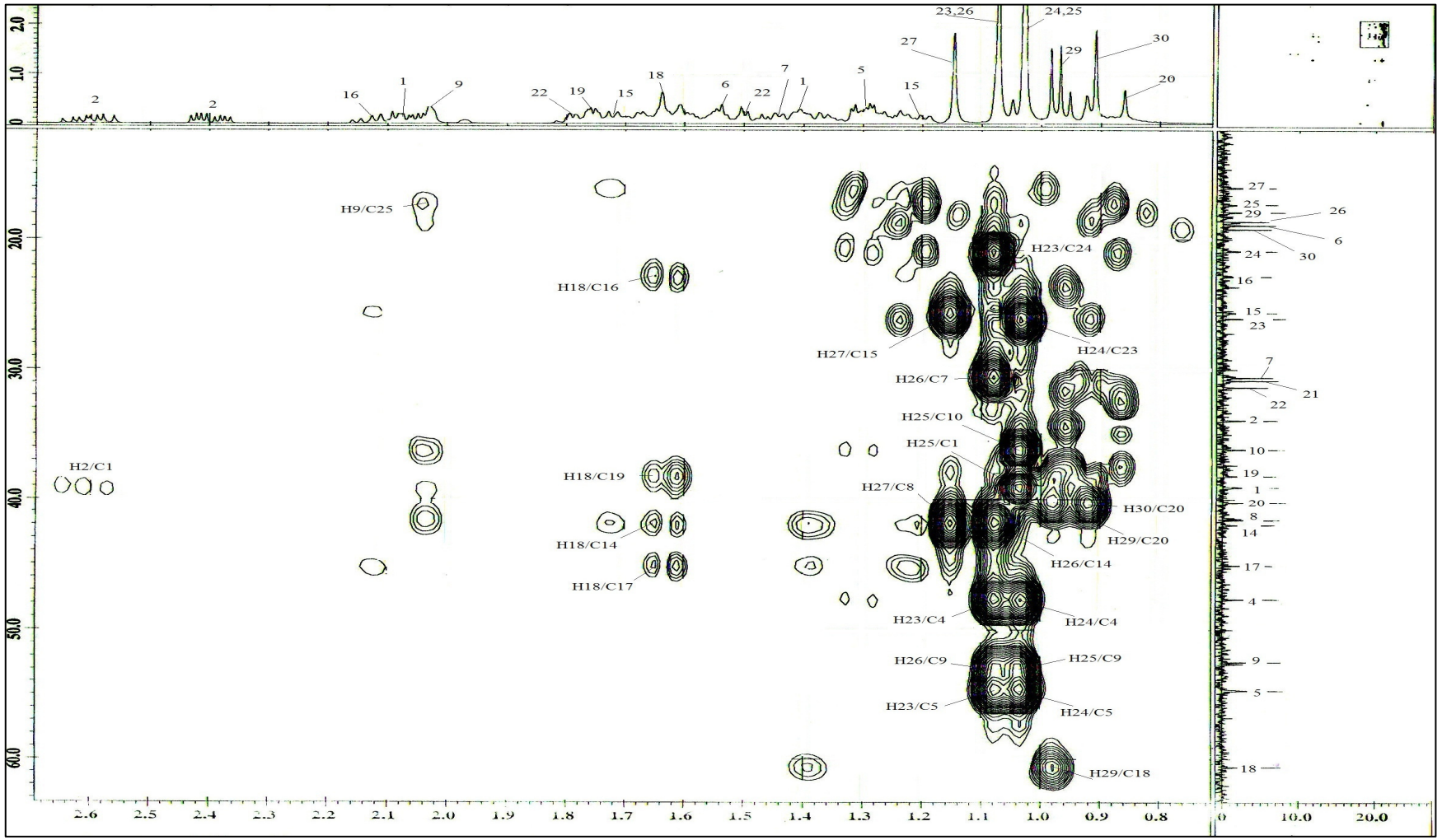
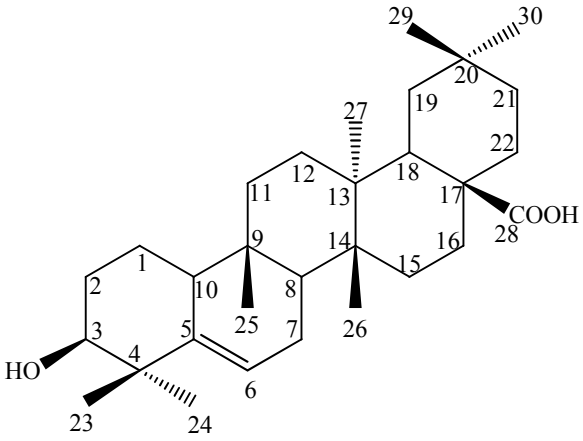


Figure 3.8.5: HMBC Spectrum of Compound H

3.9 Compound I : 3β-hydroxy-5-glutinen-28-oic acid 123



Compound I was isolated as a colourless crystals, mp 305-307^{o}C. Its IR spectrum showed hydroxyl absorption at 3430 cm^{-1}. The EIMS gave a molecular ion peak at *m/*z 454 (M^{+}), corresponding to the molecular formula $C_{30}H_{48}O_{3}$.

The ^{1}H NMR (Figure : 3.9.1) spectrum showed the presence of seven tertiary methyl groups, an olefinic proton showed a signal at δ_H5.59 corresponding to H-6 indicating the double bond between C-5 and C-6, and a broad singlet appeared at δ_H3.45 indicating the hydroxyl group at C-3.

The ^{13}C NMR (Figure 3.9.2) and DEPT (Figure 3.9.3) experiments showed seven tertiary methyls and ten methylene groups, five methines, and eight quaternary carbons, indicating these compound is a glutinane type of triterpene[57].

The resonance of C$\underline{H}_3$-23 at δ_H1.01 and C$\underline{H}_3$-24 at δ_H1.12 coupled with C-3 (δ_C76.4), the hydroxylated carbon confirmed by HMBC spectrum (Figure: 3.9.4). Moreover, the signal of methine proton H-18 [δ_H2.36 (*dd*, *J*=13.2. 3.9)] correlated to the signal at δ_C184.4 (C-28) . The presence of a double bond at C-5 and C-6 was confirmed by analysis of HMBC correlations. The coupling constants $J_{18,19}$ = 13.2Hz and $J_{18,19}$ = 3.9Hz due to the observed interaction of H-18 and H-19. The resonance of H-22 at δ_H2.28, $J_{22,21}$ = 14.6 Hz and $J_{22,21}$ =

9.2Hz due to the interaction of H-22 and H-21. The structure of Compound I was determined to be 3β-hydroxy-5-glutinen-28-oic[58]. The below assignments (Figure 3.9a) were made by 2D-NMR studies including HMQC and HMBC (Table 3.9)

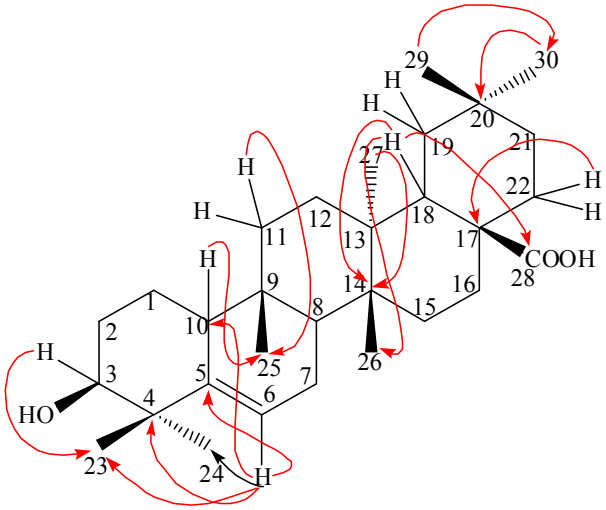


Figure 3.9a : Significant HMBC (→) interaction of Compound I

Table 3.9: ^{1}H, ^{13}C and HMBC spectral Data of Compound I in $CDCl_3$

Position	δ_C (ppm)	δ_H [ppm(*J*,Hz)]	HMBC(H→C)
1	18.4	1.38(*m*)	
		1.53(*m*)	
2	27.8	1.66(*m*)	1, 3
3	76.4	3.45(*br s*)	1, 5, 24, 25
4	40.8		
5	141.5		
6	121.8	5.59(*d*, *J*=5.8 Hz)	4, 10
7	23.5	1.16(*m*)	
		1.79(*m*)	
8	47.8	1.48(*m*)	
9	35.1		
10	49.4	2.02(*m*)	
11	34.5	1.17(*m*)	
		1.28(*m*)	
12	30.9	1.42(*m*)	
13	38.7		
14	37.2		
15	32.5	1.23(*m*)	
16	35.9	1.45(*m*)	
17	44.8		
18	37.8	2.36(*dd*, *J*=13.2, 3.9 Hz)	14, 28
19	34.9	1.50(*m*)	
20	28.5		
21	32.8	1.41(*m*)	
22	29.3	2.28(*dd*, *J*=14.6, 9.2 Hz)	17
23	29.0	1.01(*s*)	3, 4, 5, 24
24	25.5	1.12(*s*)	3, 4, 5, 23
25	15.7	0.80(*s*)	10, 11
26	20.3	0.89(*s*)	8, 18
27	18.3	0.96(*s*)	11, 14
28	184.4		18
29	34.4	0.91(*s*)	30
30	29.8	1.01(*s*)	20, 21, 29

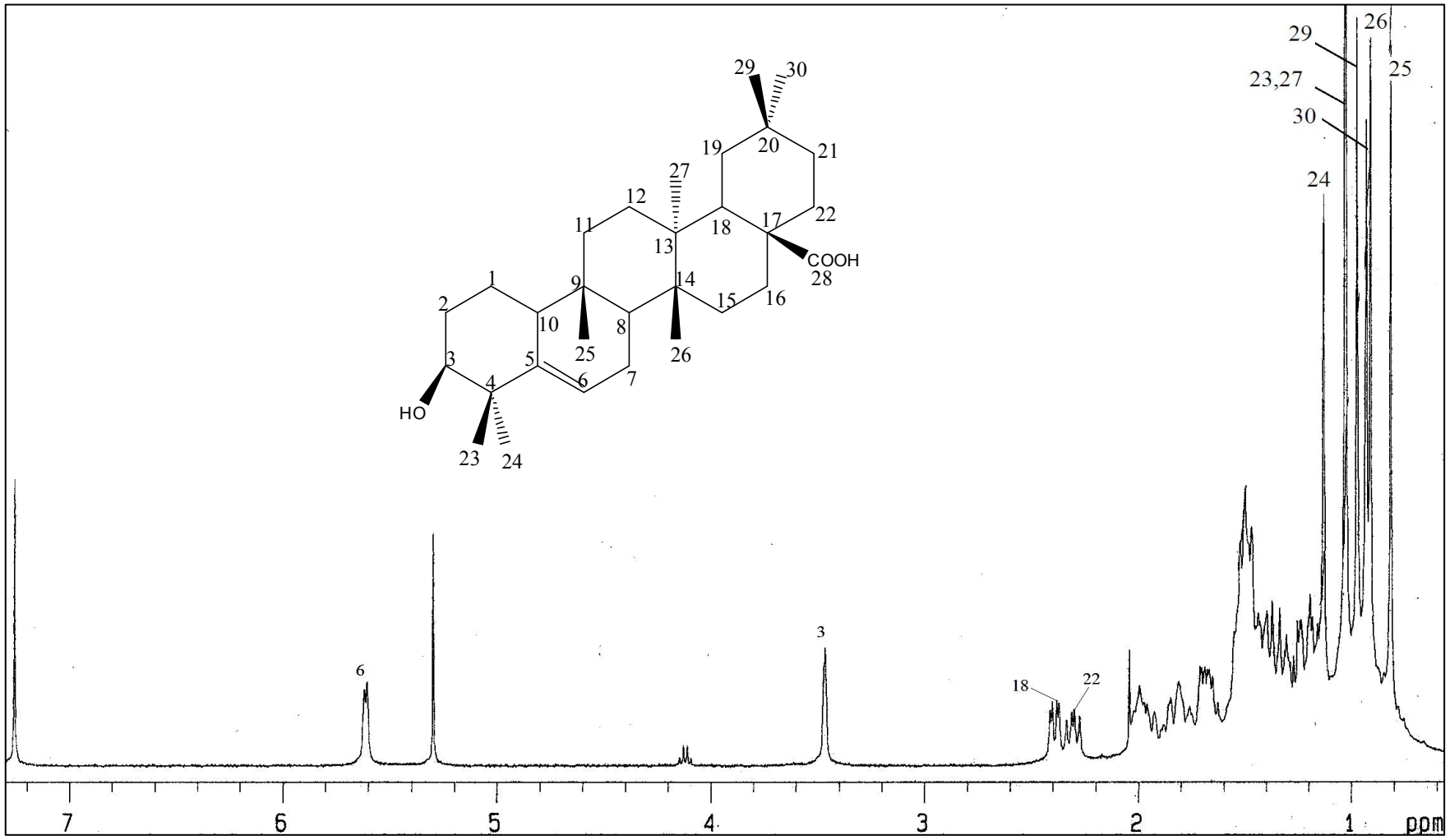


Figure 3.9.1: [1]HNMR Spectrum of Compound I

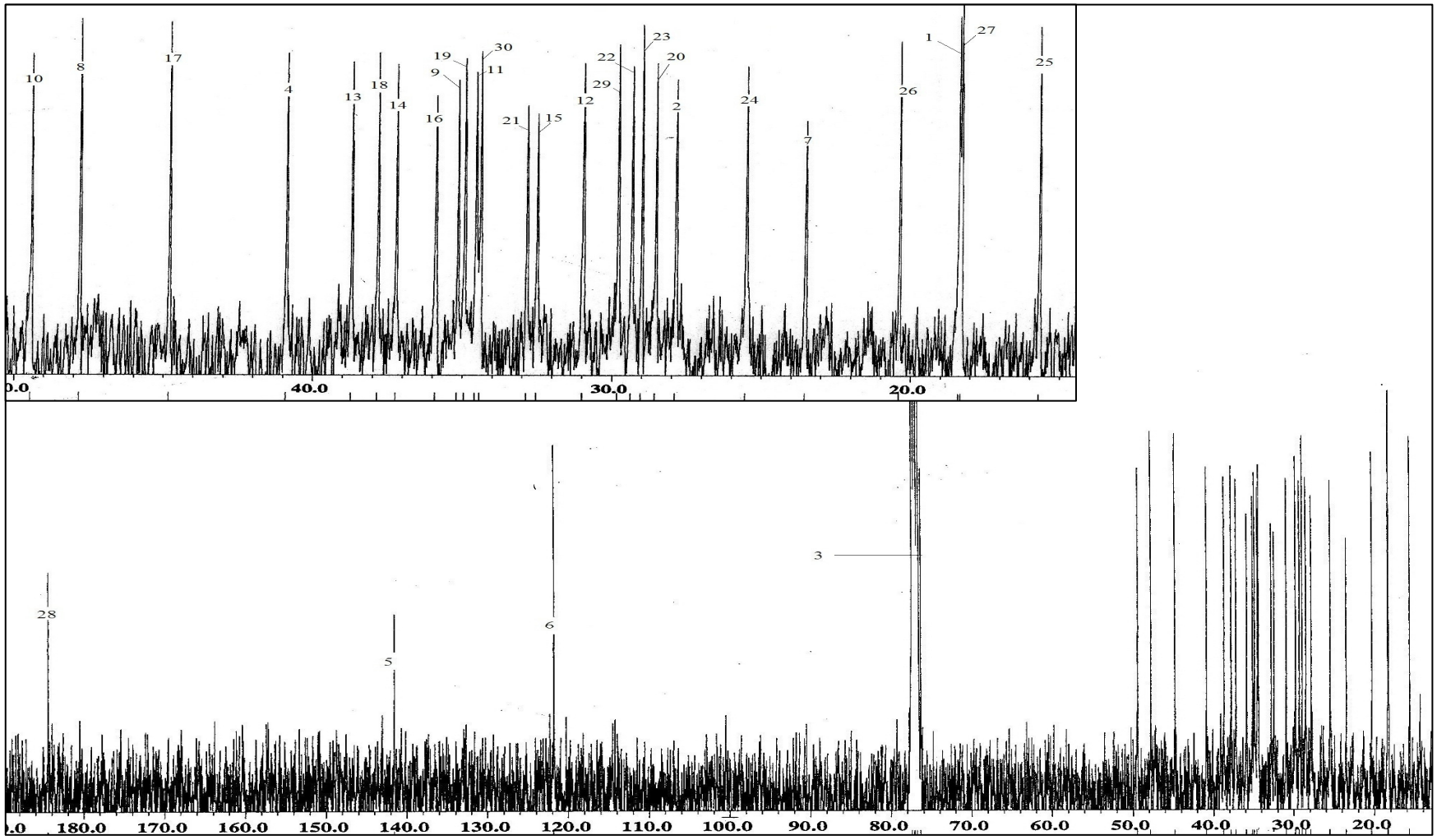


Figure 3.9.2: ^{13}CNMR Spectrum of Compound I

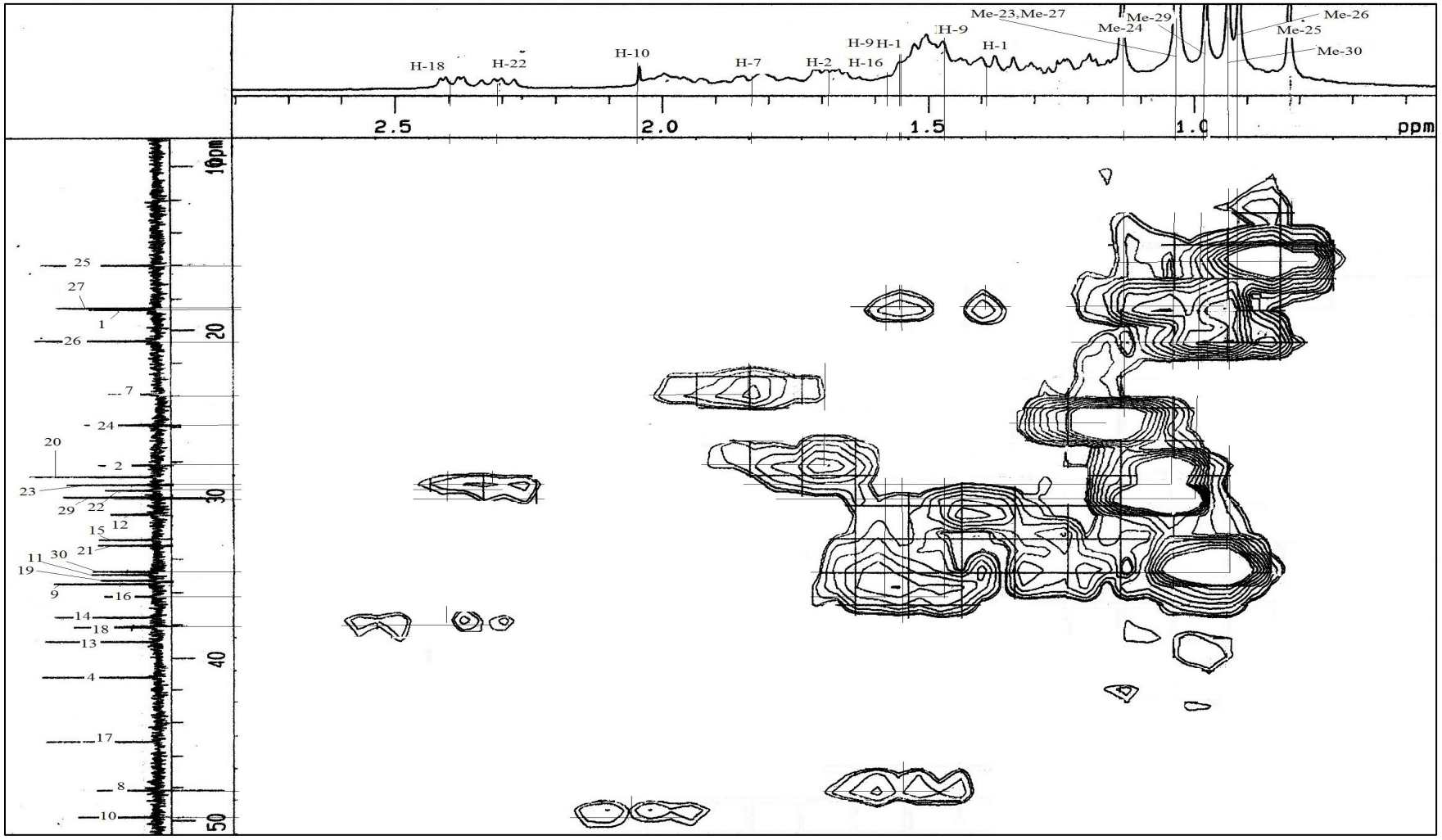


Figure 3.9.3: HSQC Spectrum of Compound I

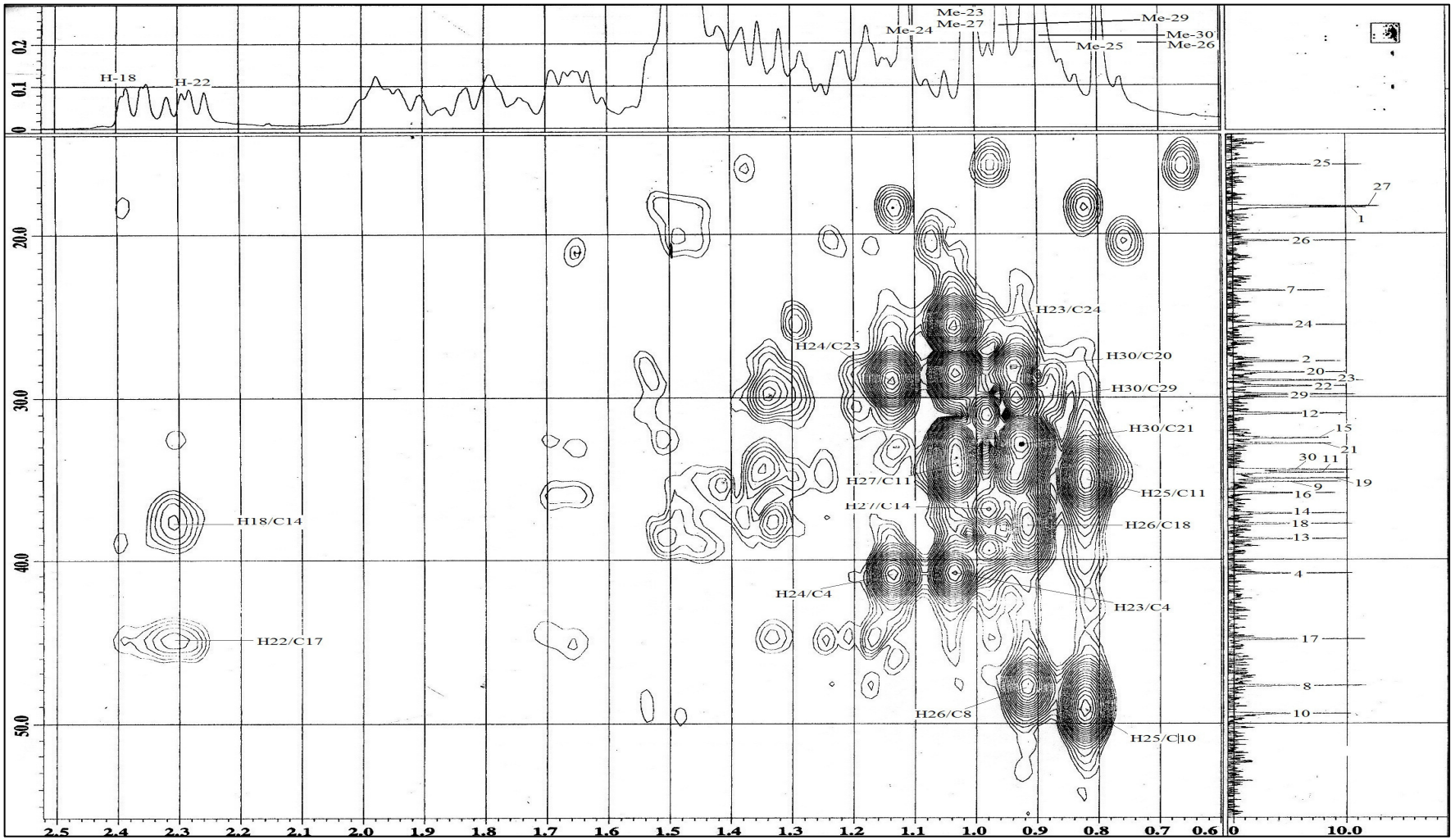


Figure 3.9.4: HMBC Spectrum of Compound I

CHAPTER 4

BIOACTIVITY

4.0 Cytotoxicity Assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a yellow tetrazolium salt that is reduced to purple formazan by dehydrogenases of a live cell. The assay is based on the principle that the amount of formazan produced is directly proportional to the number of live cells[59]. It can be used to determine cytotoxicity of potential medicinal agents.

MTT assay were performed to investigate the inhibition of cell viability and the potential cytotoxicity by crude hexane and its two pure compounds; betulonic acid (**117**) and 3*β*-hydroxy-5-glutinen-28-oic acid (**123**), on four human derived carcinoma cell lines breast (MCF-7), nasopharyngeal HSC2, cervical (CaSki) and liver (HepG2) tumor cell lines as well as in normal cell line, HMEC. The IC_{50} values were calculated in microgram per microlitre (μg/μl). HMEC was used as a control to ascertain that the compound used did not show any adverse reactions on normal cells. The IC_{50} values of both compounds betulonic acid (**117**) and 3*β*-hydroxy-5-glutinen-28-oic acid (**123**), were listed below in Table 4.1

Table 4.1 IC_{50} values of hexane extract, betulonic acid (**117**) and 3*β*-hydroxy-5-glutinen-28-oic acid (**123**) on various tumor cell lines at 24 hour incubation period.

Compound	Cell lines	IC_{50} value
Hexane Crude	HepG2	5(μg/μL)
	MCF7	6(μg/μL)
	HSC2	3(μg/μL)
	CaSki	7(μg/μL)
betulonic acid (**117**)	HepG2	19(μM)
	MCF7	12(μM)
	HSC2	13(μM)
	CaSki	14(μM)
3*β*-hydroxy-5-glutinen-28-oic acid (**123**)	HepG2	18(μM)
	MCF7	25(μM)
	HSC2	21(μM)
	CaSki	20(μM)

MCF7 and HSC2 showed highest cytotoxicity when treated with betulonic acid (**117**) at 12 μM and 13 μM respectively. While HepG2 showed the highest degree of sensitivity to 3*β*-hydroxy-5-glutinen-28-oic acid (**123**) at 18 μM.

CHAPTER 5

CONCLUSIONS

5.1 CONCLUSION

The chemical study of the hexane extract of stem bark *Walsura Pinnata* Hassk provided nine compounds by comparison of their physical and spectroscopic data with the literature values; ledol (**115**), oplopanone (**116**), betulonic acid (**117**), oleanonic acid (**118**), 3-oxoolean-11-en-13β(28)-olide (**119**), 12α-hydroxy-3-oxooleanano-28,13-lactone (**120**), 3-oxoursolic acid (**121**), 3-ketours-11-en-13β(28)-olide (**122**), and 3β-hydroxy-5-glutinen-28-oic acid (**123**). This is the first report on the chemical study of this plant species.

Compounds betulonic acid (**117**) and 3β-hydroxy-5-glutinen-28-oic acid (**123**) were tested for cytotoxicity on cancer cell lines. Both of them showed potent activity against MCF7, HSC2 and HepG2 cell lines. The IC_{50} of both compounds are 19 μM, 18 μM on HepG2; 12 μM, 25 μM on MCF-7 and 13 μM, 21 μM on HSC-2 respectively. Further works may constitute isolation and purification of dichloromethane extract and methanol extract and some mechanism investigation can be done for the cytotoxic activity betulonic acid (**117**) and 3β-hydroxy-5-glutinen-28-oic acid (**123**).

CHAPTER 6

EXPERIMENTAL

6.1 GENERAL METHODS

IR spectra were obtained using Perkin Elmer 16000 Series FT-IR spectrophotometer. Mass spectra data were recorded using Hewlett Packard HP 6890 Series Mass Selective Detector. NMR spectra were recorded using JEOL JNM-FX100 (400 MHz) and JEOL ECA 400 MHz. Chemical shifts are quoted in ppm (δ) relative to $CDCl_3$ as internal standard and coupling constants are in Hz. Column chromatography was performed with Kieselgel 60 (Merck), while TLC were performed with Kieselgel $60F_{254}$ (Merck aluminium support plates). Spots were detected under UV light or by spraying with 10% H_2SO_4 followed by heating.

6.2 CHEMICALS

Dulbecco's modified Eagle's medium (DMEM) and Roswell Park Memorial Institute-1640 (RMPI-1640) with 4.5 g glucose/L, 300 mg/l L-glutamine, 2.5% (v/v) trypsin in modified Hank's balanced salt solution (HBSS) without calcium or magnesium, fetal bovine serum (FBS) and all antibiotics were purchased from Lonza Inc., USA. MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) reagent was purchased from EMD Chemicals Inc. (Calbiochem, San Diego, CA, USA).

6.3 PLANT MATERIAL

The stem bark of *Walsura pinnata Hassk* was collected in March 1996, at 243km from Gua Musang To Kuala Lipis, Pahang, Malaysia. Identification of the plant material was done by Mr. L. E. Teo. A voucher specimen (KL 4571). has been deposited at Chemistry Department, Faculty of Science, University of Malaya.

6.4 EXTRACTION AND ISOLATION

Air-dried, powdered stem bark of *Walsura pinnata Hassk* (2.3 kg) was exhaustively extracted by soaking in *n*-hexane at room temperature for three days each for three times. The extract was filtered and separately reduced to dryness in vacuum to obtain the *n*-hexane extract (25.0 g). The *n*-hexane extract (10.0 g) was fractionated by gravity column chromatography on silica gel using *n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate-methanol and finally, pure methanol as the mobile phase to yield 10 fractions. Fraction 2 (0.7 g) was further purified by silica gel column chromatography using *n*-hexane:EtOAc (98:2) as eluent to afford pure compound **A** (2 mg) whereas fraction 5 (*n*-hexane:EtOAc, 85:15) and fraction 9 (*n*-hexane:EtOAc, 80:20) afforded pure compound **C (**10 mg) and **I** (5 mg) respectively. Fraction 6 (0.8 g) was subsequently subjected to silica gel column chromatography using *n*-hexane:acetone (96:4) as eluent to afford pure compound **E** (2 mg). Purification of fraction 7 (3.0 g) on column chromatography using *n*-hexane:acetone (94:6, 93:7, 91:9) led to isolation of compound **D** (2 mg), fraction 7-G (0.1 g) and compound **G** (2 mg) respectively. Fraction 7-G was further chromatographed successively by gravity column chromatography using *n*-hexane:acetone (95:5 and 93:7) to yield compound **H** (2 mg) and compound **F** (2mg) respectively in pure forms. Finally, fraction 10 (50 mg) was separated by CC using *n*-hexane:acetone (96:4) as eluent to give pure compound **B** (5 mg). The isolation and purification procedure was summarized in the flow chart shown in Figure 6.4a.

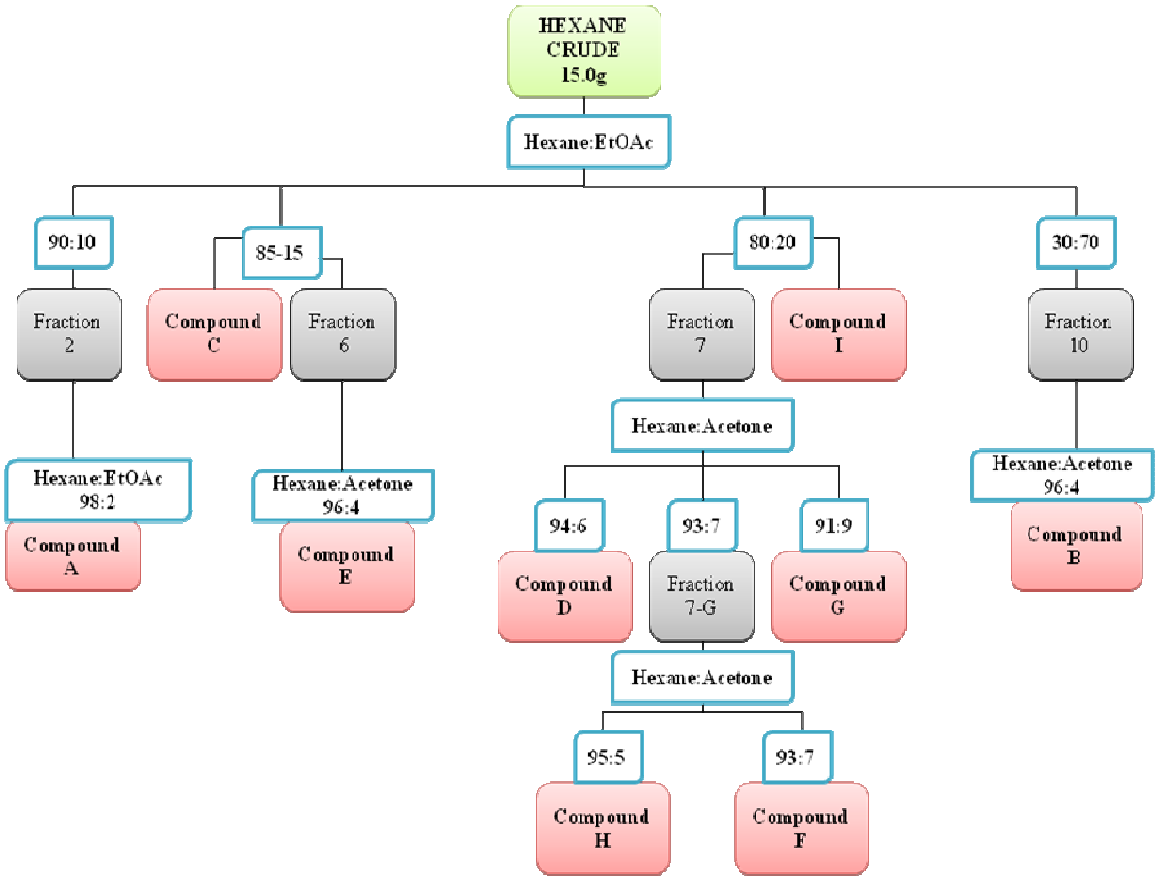


Figure 6.4a : Flow chart of Isolation and purification.

6.5 CULTIVATION OF CELLS

A total of five tumour cell lines were used in this study. Human breast adenocarcinoma (MCF-7) and oral squamous carcinoma (HSC-2 and HSC-4) were obtained from the Cancer Research Initiative Foundation (CARIF, Malaysia), while human hepatocyte carcinoma (HepG2) and epidermoid cervical carcinoma (CaSki) cells were obtained from University Malaya Medical Centre (UMMC, Malaysia). Human mammary ephithelial cells (HMEC) (Lonza, USA) were used as normal cell controls. All cells were cultured in DMEM except MCF-7 cells which were cultured as monolayers in RMPI-1640, supplemented with 10.0% (v/v) FBS, 100.0 U/ml penicillin and 100.0 µg/ml streptomycin. Cultures were maintained in a humidified CO_2 incubator at 37 ºC in 5.0% CO_2 and 95.0% air.

6.6 CELL VIABILITY ASSAY

Cell viability was determined using the MTT assay which measures mitochondrial activity in viable cells *n*-hexane crude was dissolved in DMSO to a final concentration of 10.0 mM. Briefly, $2.0x10^4$ cells were treated in triplicates on 96-well plates in the presence or absence of *n*-hexane crude at final concentrations of 5.0 μM to 80.0 μM up to 24 hours. Final DMSO concentration in each experiment was maintained below 0.05% (v/v) to prevent solvent induced cytotoxicty. 20.0 μl of MTT dye reagent (5.0 mg/ml) was added to each well and cells were incubated in the dark at 37 ºC. After 2 hours of incubation, the media containing excess dye was aspirated and 200.0 μL of DMSO was added to dissolve the purple formazan precipitates. A microtiter plate reader (Tecan Sunrise®, Switzerland) was used to detect absorbance at a test wavelength of 570 nm, with a reference wavelength of 650 nm.

6.7 SPECTRAL DATA OF ISOLATED COMPOUNDS.

Ledol 115 : gummy oil

Molecular formula : $C_{15}H_{26}O$

IR (cm^{-1}) : 3381 cm^{-1} (3º OH)

$[\alpha]_D$: +1.5º (MeOH; *c*=0.0005)

Mass : *m/z* 204

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.1

Oplopanone 116 : gummy oil

Molecular formula : $C_{15}H_{26}O_2$

IR (cm^{-1}) : 3376 cm^{-1} (OH), 1706 cm^{-1} (C=O).

Mass : *m/z* 238

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.2

Betulonic Acid 117 : amourphous

Molecular formula : $C_{30}H_{46}O_3$

IR (cm^{-1}) : 3072-2870 cm^{-1}(COOH) and 1694 cm^{-1} C=O).

$[\alpha]_D$: $+42.0^o$ (MeOH ;*c*=0.0002)

Mass : *m/z* 453

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.3

Oleanonic acid 118 : crystal mp178^{o}C [lit. mp 178-179 ^{o}C]

Molecular formula : $C_{30}H_{47}O_3$

IR (cm^{-1}) : 2947 cm^{-1} (br, COOH) and
1696 cm^{-1} (acid C=O).

Mass : *m/z* 454

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.4

3-oxoolean-11-en-13*β*(28)-olide 119: gummy oil

Molecular formula : $C_{30}H_{44}O_3$

IR (cm^{-1}) : 1765 cm^{-1} (lactone C=O), 1705 cm^{-1} (ketone C=O), and 1640 cm^{-1} (C=C).

Mass : *m/z* 452

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.5

12*α*-hydroxy-3-oxooleanano-28,13-lactone 120 :gummy oil

Molecular formula : $C_{30}H_{46}O_4$

IR (cm^{-1}) : 3619 cm^{-1} (OH), 1760 cm^{-1} (lactone C=O), 1699cm^{-1} (ketone C=O)

$[\alpha]^{23.5}_D$: +63.6° ($CHCl_3$;*c*=1.001)

Mass : *m/z* 470

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.6

3-oxoursolic acid 121 : amorphous

Molecular formula : $C_{30}H_{47}O_3$

IR (cm^{-1}) : 3400-2920 cm^{-1} (COOH), 1695 cm^{-1} (ketone C=O) and 1670 cm^{-1} (C=C).

Mass : *m/z*: 454

^1H NMR and ^{13}C NMR in $CDCl_3$: refer Table 3.7

3-ketours-11-en-13*β*(28)-olide 122 : gummy oil

Molecular formula : $C_{30}H_{44}O_3$

IR (cm^{-1}) : 2634, 2866 cm^{-1} (CH), 1765 cm^{-1} (lactone C=O), 1703 cm^{-1} (ketone C=O), and 1137 cm^{-1} (C-O).

Mass : *m/z*:452

^1H and ^{13}CNMR in $CDCl_3$: refer Table 3.8

3*β*-hydroxy-5-glutinen-28-oic acid 123 : Crystal mp 305-307^oC

Molecular formula : $C_{30}H_{48}O_3$.

IR (cm^{-1}) : 3430cm^{-1} (COOH)

$[\alpha]^{25}_D$: +40.3^o ($CHCl_3$;*c*=0.0003)

Mass : *m/*z 454

^1H NMR and ^{13}C NMR in $CDCl_3$: refer Table 3.9

CHAPTER 7

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APPENDIX

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3-Oxoolean-1-en-28-oic acid–*n*-hexane–water (4/1/1) from the bark of *Walsura pinnata* Hassk

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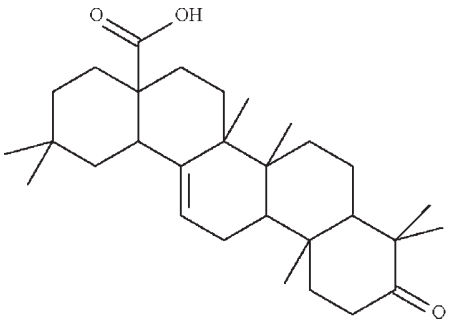
Key indicators: single-crystal X-ray study; T = 100 K; mean σ(C–C) = 0.007 Å; disorder in main residue; R factor = 0.068; wR factor = 0.204; data-to-parameter ratio = 10.4.

3-Oxoolean-1-en-28-oic acid, isolated from the bark of *Walsura pinnata* Hassk, crystallized from *n*-hexane as an *n*-hexane 0.25-solvent 0.25-hydrate, $C_{30}H_{46}O_3\cdot0.25C_6H_{14}\cdot0.25H_2O$. There are two independent molecules in the asymmetric unit of the title compound. The three six-membered cyclohexane rings in each molecule adopt chair conformations and the carboxyl substituent occupies an axial/equatorial position. The two independent molecules are linked by a pair of $O-H_{carboxyl}\cdots O$ hydrogen bonds into a dimer. The *n*-hexane molecule is disordered about a twofold rotation axis and the water molecule lies on a twofold rotation axis. In addition, the cyclohexone carbonyl group of one of the independent molecules is disordered over two sites with occupancies of 0.75 and 0.25.

Related literature

There are no reports of chemicals from *Walsura pinnata* Hassk. For the action of a fungus on this compound, isolated from another source, see: Shirane *et al.* (1996).



·1/4C_6H_{14} 1/4H_2O

Experimental

Crystal data

$C_{30}H_{46}O_3\cdot0.25C_6H_{14}\cdot0.25H_2O$
M_r = 480.71
Monoclinic, $C2$
a = 28.5864 (7) Å
b = 12.2408 (3) Å
c = 19.3545 (4) Å
β = 120.552 (1)°
V = 5832.3 (2) Å^3
Z = 8
Mo $K\alpha$ radiation
μ = 0.07 mm^{-1}
T = 100 K
0.45 × 0.15 × 0.10 mm

Data collection

Bruker SMART APEX diffractometer
Absorption correction: none
20554 measured reflections
6997 independent reflections
5441 reflections with $I > 2\sigma(I)$
R_{int} = 0.038

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.068
$wR(F^2)$ = 0.204
S = 1.07
6997 reflections
676 parameters
71 restraints
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.81 e Å^{-3}
$\Delta\rho_{min}$ = −0.62 e Å^{-3}

Table 1
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1···O4	0.84	1.71	2.545 (4)	171
O5—H5···O2	0.84	1.83	2.637 (4)	160
O1w—H1w···O3	0.84	2.20	3.03 (2)	169

Data collection: *APEX2* (Bruker, 2007); cell refinement: *SAINT* (Bruker, 2007); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *X-SEED* (Barbour, 2001); software used to prepare material for publication: *publCIF* (Westrip, 2009).

We thank the University of Malaya for supporting this study.

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: LH2809).

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supplementary materials

Acta Cryst. (2009). E**65**, o1166 [**doi:10.1107/S1600536809015086**]

3-Oxoolean-1-en-28-oic acid-*n*-hexane-water (4/1/1) from the bark of *Walsura pinnata* Hassk

K. Awang, M. Yusoff, K. Mohamad, S. L. Chong and S. W. Ng

Comment

The two independent molecules are shown in Fig. 1.

Experimental

The dried and ground bark of *Walsura pinnata* Hassk (2.3 kg) was extracted with *n*-hexane for 72 h at room temperature. The solvent was evaporated to give a crude extract, which was subjected to column chromatography on silica gel (60 GF_{254}) by using *n*-hexane with increasing amounts of ethyl acetate as eluent. Of the twenty-four fractions collected, the twenty-second fraction, eluted with ethyl acetate:*n*-hexane, 14:86 gave 2 g of the product, which was further purified by column chromatography (*n*-hexane:ethyl acetate 80:20) to give the title compound (10 mg).

The formulation was established by satisfactory solution NMR spectroscopy.

Refinement

The carbonyl group of one of the two independent molecules is disordered over two positions. For this unit – C17–C18(=O3)–C19 – the 1,2- and 1,3-related distances of the umprimed and primed atoms were restrained to within 0.01 Å of each other. The temperature factors of the umprimed atoms were set to those of the prime ones for the C18/C18' and O3/O3' atoms. The four-atom unit was restrained to be nearly planar.

The hexane molecule lies on a twofold rotation axis; the molecule was instead refined as a six-carbon species, with 1,2- and 1,3-related distances being restrained to 1.54±0.01 and 2.51±0.01 Å. The anisotropic temperature factors of the six carbon atoms were restrained to be nearly isotropic.

The water molecules lies on a twofold rotation axis; the oxygen atom showed large anisotropic temperature factors.

Carbon-bound H-atoms were placed in calculated positions (C—H 0.95–0.99 Å) and were included in the refinement in the riding model approximation, with U(H) set to 1.2–1.5U_{eq}(C). The acid H-atoms were placed in chemically sensible positions but were not refined.

In the absence of heavy scatterers, Friedel pairs were merged.

Figures

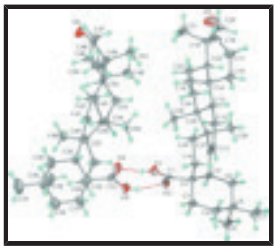


Fig. 1. 70% Probability thermal-ellipsoid plot (Barbour, 2001) of the two independent molecules of $C_{30}H_{46}O_3 \cdot 1/4C_6H_{14} \cdot 1/4H_2O$. Hydrogen atoms are drawn as spheres of arbitrary radii. The disorder in one of the cyclohexanone carbonyl groups and the solvent molecules are not shown.

3-Oxoolean-1-en-28-oic acid–*n*-hexane–water (4/1/1)

Crystal data

$C_{30}H_{46}O_3 \cdot 0.25C_6H_{14} \cdot 0.25H_2O$	$F_{000} = 2120$
$M_r = 480.71$	$D_x = 1.095$ Mg m^{-3}
Monoclinic, *C*2	Mo *K*α radiation λ = 0.71073 Å
Hall symbol: C 2y	Cell parameters from 4238 reflections
a = 28.5864 (7) Å	θ = 2.4–22.7º
b = 12.2408 (3) Å	μ = 0.07 mm^{-1}
c = 19.3545 (4) Å	*T* = 100 K
β = 120.552 (1)º	Colorless, prism
V = 5832.3 (2) Å^3	0.45 × 0.15 × 0.10 mm
Z = 8	

Data collection

Bruker SMART APEX diffractometer	5441 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	$R_{int} = 0.038$
Monochromator: graphite	$\theta_{max} = 27.5$º
T = 100 K	$\theta_{min} = 1.2$º
ω scans	*h* = −37→36
Absorption correction: None	*k* = −15→15
20554 measured reflections	*l* = −25→24
6997 independent reflections	

Refinement

Refinement on F^2	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: inferred from neighbouring sites
$R[F^2 > 2\sigma(F^2)] = 0.068$	H-atom parameters constrained
$wR(F^2) = 0.204$	$w = 1/[\sigma^2(F_o^2) + (0.1276P)^2 + 2.2067P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.07	$(\Delta/\sigma)_{max} = 0.001$

6997 reflections	$\Delta\rho_{max}$ = 0.81 e Å^{-3}
676 parameters	$\Delta\rho_{min}$ = −0.62 e Å^{-3}
71 restraints	Extinction correction: none
Primary atom site location: structure-invariant direct methods	

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}	Occ. (<1)
O1	0.64169 (14)	0.4999 (3)	0.06365 (17)	0.0372 (7)	
H1	0.6626	0.5079	0.1133	0.056*	
O2	0.64271 (12)	0.6807 (3)	0.06809 (17)	0.0338 (7)	
O3	0.9328 (2)	0.6613 (5)	−0.1228 (4)	0.0706 (16)	0.75
O3'	0.8798 (6)	0.6340 (10)	−0.2241 (7)	0.0706 (16)	0.25
O4	0.71245 (16)	0.5134 (3)	0.21228 (19)	0.0543 (10)	
O5	0.70076 (14)	0.6881 (3)	0.22605 (19)	0.0427 (8)	
H5	0.6763	0.6814	0.1777	0.064*	
O6	1.12997 (15)	0.6700 (4)	0.3873 (3)	0.0672 (12)	
O1W	1.0000	0.834 (2)	0.0000	0.248 (9)	
H1W	0.9789	0.7934	−0.0385	0.371*	
C1	0.62647 (15)	0.5947 (4)	0.0301 (2)	0.0268 (8)	
C2	0.58412 (15)	0.5936 (4)	−0.0588 (2)	0.0258 (8)	
C3	0.52936 (16)	0.5977 (4)	−0.0593 (3)	0.0338 (9)	
H3A	0.5240	0.5273	−0.0391	0.041*	
H3B	0.5314	0.6560	−0.0224	0.041*	
C4	0.48058 (17)	0.6192 (4)	−0.1430 (3)	0.0399 (11)	
H4A	0.4758	0.5562	−0.1781	0.048*	
H4B	0.4474	0.6253	−0.1392	0.048*	
C5	0.48699 (17)	0.7227 (4)	−0.1809 (3)	0.0371 (10)	
C6	0.4882 (2)	0.8240 (5)	−0.1331 (3)	0.0462 (12)	
H6A	0.4548	0.8267	−0.1305	0.069*	
H6B	0.4908	0.8899	−0.1596	0.069*	
H6C	0.5197	0.8198	−0.0786	0.069*	
C7	0.43893 (19)	0.7325 (5)	−0.2667 (3)	0.0505 (13)	
H7A	0.4051	0.7373	−0.2657	0.076*	
H7B	0.4377	0.6681	−0.2977	0.076*	
H7C	0.4432	0.7984	−0.2918	0.076*	
C8	0.54003 (16)	0.7146 (4)	−0.1828 (2)	0.0308 (9)	
H8A	0.5450	0.7833	−0.2054	0.037*	
H8B	0.5364	0.6544	−0.2193	0.037*	
C9	0.59140 (15)	0.6944 (3)	−0.1001 (2)	0.0252 (8)	
H9	0.5960	0.7592	−0.0657	0.030*	
C10	0.64277 (14)	0.6855 (3)	−0.1054 (2)	0.0213 (7)	
C11	0.67132 (16)	0.7747 (3)	−0.0975 (2)	0.0247 (8)	
H11	0.6585	0.8412	−0.0878	0.030*	
C12	0.72209 (16)	0.7808 (3)	−0.1023 (2)	0.0265 (8)	
H12A	0.7522	0.8074	−0.0503	0.032*	
H12B	0.7165	0.8349	−0.1439	0.032*	

C13	0.73895 (15)	0.6714 (3)	−0.1223 (2)	0.0215 (7)	
H13	0.7143	0.6629	−0.1815	0.026*	
C14	0.79755 (15)	0.6718 (3)	−0.1098 (2)	0.0248 (8)	
C15	0.84201 (17)	0.7066 (4)	−0.0258 (3)	0.0335 (9)	
H15A	0.8762	0.7167	−0.0252	0.050*	
H15B	0.8467	0.6500	0.0130	0.050*	
H15C	0.8316	0.7755	−0.0115	0.050*	
C16	0.79644 (18)	0.7532 (4)	−0.1717 (3)	0.0363 (10)	
H16A	0.7663	0.7334	−0.2258	0.044*	
H16B	0.7892	0.8274	−0.1591	0.044*	
C17	0.8498 (2)	0.7548 (4)	−0.1727 (3)	0.0431 (12)	
H17A	0.8412	0.7684	−0.2284	0.052*	
H17B	0.8722	0.8166	−0.1394	0.052*	
C18	0.8821 (3)	0.6541 (6)	−0.1435 (3)	0.0526 (19)	0.75
C18'	0.8654 (3)	0.6426 (8)	−0.1818 (6)	0.0526 (19)	0.25
C19	0.86193 (19)	0.5453 (4)	−0.1348 (3)	0.0376 (10)	
C20	0.8513 (2)	0.4719 (6)	−0.2056 (3)	0.0541 (15)	
H20A	0.8854	0.4606	−0.2053	0.081*	
H20B	0.8247	0.5070	−0.2560	0.081*	
H20C	0.8371	0.4013	−0.2007	0.081*	
C21	0.9086 (2)	0.4917 (8)	−0.0581 (3)	0.070 (2)	
H21A	0.9407	0.4844	−0.0636	0.104*	
H21B	0.8972	0.4193	−0.0507	0.104*	
H21C	0.9175	0.5376	−0.0115	0.104*	
C22	0.80785 (16)	0.5581 (3)	−0.1335 (2)	0.0257 (8)	
H22	0.7780	0.5468	−0.1902	0.031*	
C23	0.80078 (17)	0.4670 (3)	−0.0851 (2)	0.0291 (8)	
H23A	0.8098	0.3956	−0.0993	0.035*	
H23B	0.8258	0.4795	−0.0270	0.035*	
C24	0.74204 (17)	0.4658 (3)	−0.1034 (2)	0.0273 (8)	
H24A	0.7381	0.4075	−0.0712	0.033*	
H24B	0.7177	0.4476	−0.1607	0.033*	
C25	0.72373 (15)	0.5751 (3)	−0.0854 (2)	0.0225 (7)	
C26	0.75144 (15)	0.5890 (4)	0.0067 (2)	0.0259 (8)	
H26A	0.7907	0.5791	0.0312	0.039*	
H26B	0.7371	0.5343	0.0281	0.039*	
H26C	0.7440	0.6623	0.0190	0.039*	
C27	0.65940 (15)	0.5761 (3)	−0.1251 (2)	0.0225 (7)	
C28	0.62911 (16)	0.5670 (3)	−0.2184 (2)	0.0275 (8)	
H28A	0.5908	0.5494	−0.2389	0.041*	
H28B	0.6458	0.5091	−0.2338	0.041*	
H28C	0.6317	0.6366	−0.2412	0.041*	
C29	0.64230 (16)	0.4771 (3)	−0.0940 (2)	0.0251 (8)	
H29A	0.6412	0.4117	−0.1248	0.030*	
H29B	0.6704	0.4643	−0.0372	0.030*	
C30	0.58735 (16)	0.4889 (3)	−0.0997 (2)	0.0259 (8)	
H30A	0.5811	0.4250	−0.0741	0.031*	
H30B	0.5583	0.4900	−0.1569	0.031*	
C31	0.72161 (17)	0.5932 (4)	0.2551 (2)	0.0339 (9)	

C32	0.75531 (17)	0.5834 (4)	0.3459 (2)	0.0339 (9)
C33	0.7125 (2)	0.5534 (5)	0.3692 (3)	0.0433 (11)
H33A	0.6899	0.6185	0.3623	0.052*
H33B	0.6883	0.4959	0.3324	0.052*
C34	0.7382 (2)	0.5129 (6)	0.4555 (3)	0.0551 (15)
H34A	0.7090	0.4893	0.4655	0.066*
H34B	0.7578	0.5741	0.4924	0.066*
C35	0.7776 (2)	0.4181 (6)	0.4742 (3)	0.0597 (17)
C36	0.7479 (3)	0.3152 (6)	0.4269 (4)	0.071 (2)
H36A	0.7736	0.2542	0.4438	0.106*
H36B	0.7326	0.3285	0.3694	0.106*
H36C	0.7186	0.2972	0.4372	0.106*
C37	0.8052 (3)	0.3955 (8)	0.5653 (4)	0.084 (3)
H37A	0.8302	0.3336	0.5795	0.126*
H37B	0.7775	0.3782	0.5791	0.126*
H37C	0.8255	0.4604	0.5952	0.126*
C38	0.8213 (2)	0.4554 (5)	0.4546 (3)	0.0468 (13)
H38A	0.8422	0.5163	0.4907	0.056*
H38B	0.8468	0.3942	0.4654	0.056*
C39	0.79764 (18)	0.4926 (4)	0.3673 (2)	0.0359 (10)
H39	0.7778	0.4285	0.3327	0.043*
C40	0.84081 (17)	0.5249 (4)	0.3471 (2)	0.0314 (9)
C41	0.85843 (19)	0.4506 (4)	0.3156 (3)	0.0413 (11)
H41	0.8428	0.3798	0.3068	0.050*
C42	0.9010 (2)	0.4683 (5)	0.2927 (3)	0.0489 (13)
H42A	0.8839	0.4606	0.2338	0.059*
H42B	0.9290	0.4105	0.3179	0.059*
C43	0.92871 (16)	0.5796 (4)	0.3176 (2)	0.0305 (9)
H43	0.9536	0.5741	0.3770	0.037*
C44	0.96696 (16)	0.6060 (4)	0.2835 (2)	0.0329 (10)
C45	0.9375 (2)	0.6018 (6)	0.1903 (3)	0.0561 (16)
H45A	0.9182	0.6707	0.1680	0.084*
H45B	0.9114	0.5413	0.1706	0.084*
H45C	0.9643	0.5906	0.1736	0.084*
C46	1.01230 (18)	0.5192 (4)	0.3172 (3)	0.0396 (11)
H46A	1.0265	0.5103	0.3754	0.048*
H46B	0.9969	0.4483	0.2908	0.048*
C47	1.0592 (2)	0.5498 (5)	0.3042 (3)	0.0451 (12)
H47A	1.0879	0.4935	0.3293	0.054*
H47B	1.0459	0.5506	0.2460	0.054*
C48	1.08275 (19)	0.6585 (5)	0.3391 (3)	0.0419 (11)
C49	1.0432 (2)	0.7552 (4)	0.3119 (3)	0.0423 (11)
C50	1.0716 (3)	0.8501 (5)	0.3670 (5)	0.0698 (19)
H50A	1.0748	0.8366	0.4191	0.105*
H50B	1.0505	0.9170	0.3435	0.105*
H50C	1.1079	0.8586	0.3742	0.105*
C51	1.0297 (3)	0.7881 (7)	0.2266 (4)	0.073 (2)
H51A	1.0634	0.8057	0.2273	0.109*
H51B	1.0059	0.8522	0.2089	0.109*

H51C	1.0114	0.7273	0.1896	0.109*	
C52	0.99266 (18)	0.7183 (4)	0.3177 (2)	0.0339 (10)	
H52	1.0073	0.7109	0.3766	0.041*	
C53	0.9495 (2)	0.8059 (4)	0.2916 (3)	0.0425 (12)	
H53A	0.9273	0.8078	0.2323	0.051*	
H53B	0.9670	0.8782	0.3104	0.051*	
C54	0.9129 (2)	0.7824 (4)	0.3265 (3)	0.0370 (10)	
H54A	0.8839	0.8383	0.3062	0.044*	
H54B	0.9348	0.7901	0.3855	0.044*	
C55	0.88640 (17)	0.6687 (4)	0.3064 (2)	0.0302 (9)	
C56	0.83882 (18)	0.6701 (5)	0.2192 (2)	0.0452 (13)	
H56A	0.8526	0.6849	0.1830	0.068*	
H56B	0.8130	0.7274	0.2130	0.068*	
H56C	0.8205	0.5991	0.2060	0.068*	
C57	0.86480 (17)	0.6396 (4)	0.3657 (2)	0.0298 (9)	
C58	0.91077 (17)	0.6397 (4)	0.4546 (2)	0.0330 (9)	
H58A	0.8949	0.6390	0.4891	0.050*	
H58B	0.9331	0.7054	0.4656	0.050*	
H58C	0.9335	0.5747	0.4654	0.050*	
C59	0.82326 (18)	0.7272 (4)	0.3573 (3)	0.0332 (9)	
H59A	0.8433	0.7924	0.3887	0.040*	
H59B	0.8016	0.7494	0.3003	0.040*	
C60	0.78427 (18)	0.6904 (4)	0.3852 (3)	0.0359 (10)	
H60A	0.8049	0.6809	0.4442	0.043*	
H60B	0.7568	0.7482	0.3724	0.043*	
C61	0.8146 (6)	1.0554 (15)	0.3756 (8)	0.092 (5)	0.50
H61A	0.7983	0.9941	0.3382	0.138*	0.50
H61B	0.8207	1.1164	0.3483	0.138*	0.50
H61C	0.7900	1.0786	0.3941	0.138*	0.50
C63	0.8949 (7)	1.1153 (17)	0.5058 (10)	0.139 (8)	0.50
H63A	0.9253	1.0880	0.5573	0.167*	0.50
H63B	0.8683	1.1517	0.5163	0.167*	0.50
C64	0.9164 (11)	1.197 (2)	0.4664 (11)	0.179 (12)	0.50
H64A	0.9315	1.1571	0.4376	0.215*	0.50
H64B	0.8866	1.2449	0.4277	0.215*	0.50
C62	0.8679 (7)	1.0202 (13)	0.4464 (10)	0.128 (7)	0.50
H62A	0.8924	0.9947	0.4275	0.153*	0.50
H62B	0.8618	0.9585	0.4739	0.153*	0.50
C65	0.9612 (9)	1.2658 (19)	0.5355 (12)	0.142 (9)	0.50
H65A	0.9458	1.3053	0.5640	0.170*	0.50
H65B	0.9905	1.2172	0.5743	0.170*	0.50
C66	0.9844 (11)	1.347 (2)	0.5009 (18)	0.160 (12)	0.50
H66A	1.0132	1.3901	0.5446	0.240*	0.50
H66B	0.9554	1.3966	0.4636	0.240*	0.50
H66C	0.9992	1.3079	0.4723	0.240*	0.50

Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0479 (19)	0.0291 (17)	0.0274 (14)	0.0008 (14)	0.0138 (14)	−0.0006 (13)
O2	0.0416 (17)	0.0257 (15)	0.0323 (14)	0.0010 (13)	0.0173 (13)	−0.0036 (13)
O3	0.050 (3)	0.062 (3)	0.116 (4)	0.006 (3)	0.054 (3)	0.021 (3)
O3'	0.050 (3)	0.062 (3)	0.116 (4)	0.006 (3)	0.054 (3)	0.021 (3)
O4	0.064 (2)	0.038 (2)	0.0313 (16)	0.0119 (18)	0.0029 (16)	−0.0100 (15)
O5	0.049 (2)	0.0372 (19)	0.0327 (15)	0.0086 (16)	0.0144 (14)	−0.0053 (14)
O6	0.037 (2)	0.050 (2)	0.083 (3)	−0.0008 (18)	0.0078 (19)	0.010 (2)
O1W	0.249 (12)	0.228 (13)	0.248 (12)	0.000	0.113 (9)	0.000
C1	0.0260 (18)	0.027 (2)	0.0327 (18)	0.0002 (17)	0.0190 (16)	−0.0016 (18)
C2	0.0213 (17)	0.028 (2)	0.0282 (17)	−0.0006 (16)	0.0130 (14)	−0.0018 (17)
C3	0.0272 (19)	0.039 (2)	0.041 (2)	−0.0017 (19)	0.0220 (17)	−0.003 (2)
C4	0.0219 (19)	0.046 (3)	0.048 (2)	0.0007 (19)	0.0152 (18)	−0.006 (2)
C5	0.0222 (19)	0.040 (3)	0.041 (2)	0.0043 (19)	0.0099 (18)	−0.001 (2)
C6	0.031 (2)	0.044 (3)	0.059 (3)	0.009 (2)	0.020 (2)	−0.004 (2)
C7	0.026 (2)	0.051 (3)	0.050 (3)	0.001 (2)	0.002 (2)	−0.001 (2)
C8	0.0259 (19)	0.030 (2)	0.0295 (18)	0.0019 (17)	0.0092 (16)	−0.0008 (17)
C9	0.0223 (18)	0.025 (2)	0.0264 (17)	0.0028 (16)	0.0110 (15)	−0.0024 (16)
C10	0.0214 (17)	0.0225 (19)	0.0170 (15)	0.0055 (15)	0.0074 (13)	0.0003 (14)
C11	0.0284 (19)	0.0195 (19)	0.0262 (17)	0.0072 (16)	0.0138 (15)	0.0011 (15)
C12	0.0295 (19)	0.020 (2)	0.0328 (19)	−0.0033 (17)	0.0182 (17)	−0.0036 (16)
C13	0.0237 (17)	0.0172 (17)	0.0238 (16)	0.0019 (15)	0.0121 (14)	0.0024 (15)
C14	0.0228 (17)	0.0236 (19)	0.0287 (18)	0.0016 (16)	0.0136 (15)	0.0042 (16)
C15	0.0264 (19)	0.031 (2)	0.036 (2)	−0.0015 (18)	0.0104 (17)	−0.0014 (18)
C16	0.031 (2)	0.034 (2)	0.047 (2)	0.0059 (19)	0.022 (2)	0.017 (2)
C17	0.039 (2)	0.043 (3)	0.057 (3)	0.005 (2)	0.030 (2)	0.019 (2)
C18	0.074 (5)	0.045 (3)	0.071 (5)	−0.004 (3)	0.060 (4)	−0.006 (4)
C18'	0.074 (5)	0.045 (3)	0.071 (5)	−0.004 (3)	0.060 (4)	−0.006 (4)
C19	0.043 (2)	0.037 (2)	0.048 (3)	0.005 (2)	0.034 (2)	0.003 (2)
C20	0.044 (3)	0.074 (4)	0.049 (3)	0.021 (3)	0.027 (2)	−0.009 (3)
C21	0.035 (3)	0.123 (6)	0.052 (3)	0.021 (3)	0.023 (2)	−0.004 (4)
C22	0.0254 (18)	0.028 (2)	0.0261 (18)	0.0005 (16)	0.0147 (15)	0.0033 (15)
C23	0.035 (2)	0.023 (2)	0.035 (2)	0.0045 (18)	0.0221 (18)	0.0053 (17)
C24	0.034 (2)	0.0180 (18)	0.036 (2)	0.0050 (17)	0.0220 (17)	0.0046 (16)
C25	0.0258 (17)	0.0184 (18)	0.0254 (17)	0.0012 (15)	0.0144 (14)	0.0009 (15)
C26	0.0252 (17)	0.029 (2)	0.0246 (16)	0.0035 (17)	0.0132 (14)	0.0060 (16)
C27	0.0264 (17)	0.0184 (18)	0.0245 (17)	0.0018 (16)	0.0142 (14)	−0.0009 (15)
C28	0.0300 (19)	0.027 (2)	0.0245 (17)	−0.0022 (17)	0.0135 (15)	−0.0049 (16)
C29	0.032 (2)	0.0163 (18)	0.0310 (19)	−0.0024 (16)	0.0189 (17)	−0.0027 (15)
C30	0.028 (2)	0.025 (2)	0.0266 (17)	−0.0041 (16)	0.0152 (16)	−0.0007 (16)
C31	0.036 (2)	0.032 (2)	0.0308 (19)	0.001 (2)	0.0149 (17)	−0.0063 (19)
C32	0.034 (2)	0.040 (3)	0.0271 (18)	−0.006 (2)	0.0146 (16)	−0.0055 (19)
C33	0.039 (2)	0.053 (3)	0.040 (2)	−0.011 (2)	0.022 (2)	−0.006 (2)
C34	0.048 (3)	0.079 (4)	0.038 (2)	−0.025 (3)	0.022 (2)	−0.003 (3)
C35	0.044 (3)	0.077 (4)	0.040 (3)	−0.032 (3)	0.009 (2)	0.009 (3)

C36	0.049 (3)	0.068 (4)	0.071 (4)	−0.024 (3)	0.012 (3)	0.018 (3)
C37	0.059 (4)	0.125 (7)	0.051 (3)	−0.041 (4)	0.016 (3)	0.030 (4)
C38	0.037 (2)	0.053 (3)	0.036 (2)	−0.018 (2)	0.008 (2)	0.008 (2)
C39	0.030 (2)	0.038 (3)	0.0291 (19)	−0.0081 (19)	0.0073 (17)	0.0003 (19)
C40	0.0247 (19)	0.033 (2)	0.0253 (18)	0.0014 (18)	0.0048 (16)	−0.0008 (17)
C41	0.033 (2)	0.030 (2)	0.050 (3)	−0.0028 (19)	0.014 (2)	−0.013 (2)
C42	0.035 (2)	0.042 (3)	0.063 (3)	−0.002 (2)	0.020 (2)	−0.025 (3)
C43	0.0274 (19)	0.034 (2)	0.0240 (17)	0.0042 (18)	0.0086 (15)	−0.0036 (17)
C44	0.0255 (19)	0.043 (3)	0.0225 (17)	0.0096 (19)	0.0070 (15)	0.0010 (18)
C45	0.034 (2)	0.100 (5)	0.029 (2)	0.013 (3)	0.0128 (18)	−0.012 (3)
C46	0.029 (2)	0.043 (3)	0.038 (2)	0.006 (2)	0.0109 (18)	−0.010 (2)
C47	0.031 (2)	0.048 (3)	0.051 (3)	0.011 (2)	0.017 (2)	−0.002 (2)
C48	0.037 (2)	0.049 (3)	0.039 (2)	0.010 (2)	0.019 (2)	0.012 (2)
C49	0.043 (3)	0.048 (3)	0.044 (2)	0.015 (2)	0.027 (2)	0.021 (2)
C50	0.074 (4)	0.038 (3)	0.126 (6)	−0.005 (3)	0.072 (4)	0.003 (3)
C51	0.064 (4)	0.105 (6)	0.070 (4)	0.044 (4)	0.049 (3)	0.058 (4)
C52	0.034 (2)	0.042 (3)	0.0283 (19)	0.012 (2)	0.0177 (17)	0.0119 (18)
C53	0.044 (3)	0.045 (3)	0.046 (3)	0.019 (2)	0.028 (2)	0.023 (2)
C54	0.046 (3)	0.032 (2)	0.044 (2)	0.014 (2)	0.031 (2)	0.012 (2)
C55	0.032 (2)	0.036 (2)	0.0225 (17)	0.0056 (19)	0.0133 (16)	0.0011 (17)
C56	0.034 (2)	0.075 (4)	0.0233 (18)	0.019 (2)	0.0122 (17)	0.006 (2)
C57	0.031 (2)	0.035 (2)	0.0225 (17)	0.0000 (18)	0.0128 (16)	−0.0034 (16)
C58	0.032 (2)	0.043 (2)	0.0237 (18)	−0.0104 (19)	0.0139 (16)	−0.0022 (17)
C59	0.035 (2)	0.034 (2)	0.036 (2)	−0.0038 (19)	0.0217 (18)	−0.0114 (18)
C60	0.034 (2)	0.041 (3)	0.036 (2)	−0.004 (2)	0.0209 (18)	−0.010 (2)
C61	0.119 (9)	0.086 (9)	0.066 (7)	0.030 (7)	0.044 (7)	−0.007 (6)
C63	0.130 (11)	0.126 (12)	0.155 (12)	0.005 (9)	0.067 (9)	−0.010 (9)
C64	0.182 (15)	0.173 (16)	0.178 (15)	0.007 (10)	0.089 (11)	0.008 (10)
C62	0.140 (11)	0.131 (12)	0.113 (10)	0.002 (9)	0.065 (9)	−0.018 (9)
C65	0.135 (12)	0.124 (12)	0.142 (12)	0.000 (9)	0.053 (9)	−0.013 (9)
C66	0.157 (16)	0.163 (14)	0.161 (13)	−0.013 (9)	0.081 (10)	0.006 (10)

Geometric parameters (Å, °)

O1—C1	1.292 (5)	C33—C34	1.527 (7)
O1—H1	0.8400	C33—H33A	0.9900
O2—C1	1.233 (5)	C33—H33B	0.9900
O3—C18	1.294 (8)	C34—C35	1.528 (10)
O3'—C18'	1.091 (15)	C34—H34A	0.9900
O4—C31	1.220 (6)	C34—H34B	0.9900
O5—C31	1.297 (6)	C35—C36	1.534 (9)
O5—H5	0.8400	C35—C38	1.546 (7)
O6—C48	1.197 (6)	C35—C37	1.548 (8)
O1W—H1W	0.8399	C36—H36A	0.9800
C1—C2	1.519 (5)	C36—H36B	0.9800
C2—C30	1.533 (6)	C36—H36C	0.9800
C2—C9	1.540 (6)	C37—H37A	0.9800
C2—C3	1.562 (5)	C37—H37B	0.9800
C3—C4	1.530 (6)	C37—H37C	0.9800

C3—H3A	0.9900	C38—C39	1.535 (6)
C3—H3B	0.9900	C38—H38A	0.9900
C4—C5	1.521 (7)	C38—H38B	0.9900
C4—H4A	0.9900	C39—C40	1.525 (6)
C4—H4B	0.9900	C39—H39	1.0000
C5—C7	1.531 (6)	C40—C41	1.327 (6)
C5—C6	1.537 (7)	C40—C57	1.524 (6)
C5—C8	1.538 (6)	C41—C42	1.510 (7)
C6—H6A	0.9800	C41—H41	0.9500
C6—H6B	0.9800	C42—C43	1.525 (7)
C6—H6C	0.9800	C42—H42A	0.9900
C7—H7A	0.9800	C42—H42B	0.9900
C7—H7B	0.9800	C43—C55	1.560 (6)
C7—H7C	0.9800	C43—C44	1.572 (6)
C8—C9	1.546 (5)	C43—H43	1.0000
C8—H8A	0.9900	C44—C46	1.541 (6)
C8—H8B	0.9900	C44—C52	1.540 (7)
C9—C10	1.528 (5)	C44—C45	1.556 (6)
C9—H9	1.0000	C45—H45A	0.9800
C10—C11	1.325 (6)	C45—H45B	0.9800
C10—C27	1.532 (5)	C45—H45C	0.9800
C11—C12	1.503 (5)	C46—C47	1.529 (7)
C11—H11	0.9500	C46—H46A	0.9900
C12—C13	1.537 (5)	C46—H46B	0.9900
C12—H12A	0.9900	C47—C48	1.490 (8)
C12—H12B	0.9900	C47—H47A	0.9900
C13—C25	1.550 (5)	C47—H47B	0.9900
C13—C14	1.566 (5)	C48—C49	1.533 (7)
C13—H13	1.0000	C49—C50	1.504 (9)
C14—C15	1.530 (6)	C49—C51	1.545 (7)
C14—C22	1.540 (6)	C49—C52	1.571 (6)
C14—C16	1.545 (6)	C50—H50A	0.9800
C15—H15A	0.9800	C50—H50B	0.9800
C15—H15B	0.9800	C50—H50C	0.9800
C15—H15C	0.9800	C51—H51A	0.9800
C16—C17	1.535 (6)	C51—H51B	0.9800
C16—H16A	0.9900	C51—H51C	0.9800
C16—H16B	0.9900	C52—C53	1.515 (6)
C17—C18	1.471 (9)	C52—H52	1.0000
C17—C18'	1.481 (12)	C53—C54	1.532 (6)
C17—H17A	0.9900	C53—H53A	0.9900
C17—H17B	0.9900	C53—H53B	0.9900
C18—C19	1.495 (8)	C54—C55	1.538 (7)
C18'—C19	1.533 (12)	C54—H54A	0.9900
C19—C20	1.535 (7)	C54—H54B	0.9900
C19—C21	1.550 (8)	C55—C56	1.537 (5)
C19—C22	1.566 (6)	C55—C57	1.596 (5)
C20—H20A	0.9800	C56—H56A	0.9800
C20—H20B	0.9800	C56—H56B	0.9800

C20—H20C	0.9800	C56—H56C	0.9800
C21—H21A	0.9800	C57—C59	1.546 (6)
C21—H21B	0.9800	C57—C58	1.546 (5)
C21—H21C	0.9800	C58—H58A	0.9800
C22—C23	1.534 (5)	C58—H58B	0.9800
C22—H22	1.0000	C58—H58C	0.9800
C23—C24	1.530 (6)	C59—C60	1.533 (6)
C23—H23A	0.9900	C59—H59A	0.9900
C23—H23B	0.9900	C59—H59B	0.9900
C24—C25	1.540 (5)	C60—H60A	0.9900
C24—H24A	0.9900	C60—H60B	0.9900
C24—H24B	0.9900	C61—C62	1.504 (10)
C25—C26	1.548 (5)	C61—H61A	0.9800
C25—C27	1.592 (5)	C61—H61B	0.9800
C26—H26A	0.9800	C61—H61C	0.9800
C26—H26B	0.9800	C63—C62	1.540 (10)
C26—H26C	0.9800	C63—C64	1.560 (11)
C27—C29	1.540 (5)	C63—H63A	0.9900
C27—C28	1.560 (5)	C63—H63B	0.9900
C28—H28A	0.9800	C64—C65	1.548 (11)
C28—H28B	0.9800	C64—H64A	0.9900
C28—H28C	0.9800	C64—H64B	0.9900
C29—C30	1.525 (5)	C62—H62A	0.9900
C29—H29A	0.9900	C62—H62B	0.9900
C29—H29B	0.9900	C65—C66	1.527 (11)
C30—H30A	0.9900	C65—H65A	0.9900
C30—H30B	0.9900	C65—H65B	0.9900
C31—C32	1.519 (6)	C66—H66A	0.9800
C32—C60	1.530 (7)	C66—H66B	0.9800
C32—C39	1.537 (7)	C66—H66C	0.9800
C32—C33	1.548 (6)		
C1—O1—H1	109.5	C34—C33—H33B	109.1
C31—O5—H5	109.5	C32—C33—H33B	109.1
O2—C1—O1	122.6 (3)	H33A—C33—H33B	107.8
O2—C1—C2	121.6 (4)	C33—C34—C35	113.1 (5)
O1—C1—C2	115.7 (4)	C33—C34—H34A	109.0
C1—C2—C30	111.6 (3)	C35—C34—H34A	109.0
C1—C2—C9	109.9 (3)	C33—C34—H34B	109.0
C30—C2—C9	110.1 (3)	C35—C34—H34B	109.0
C1—C2—C3	103.1 (3)	H34A—C34—H34B	107.8
C30—C2—C3	110.3 (3)	C34—C35—C36	111.4 (5)
C9—C2—C3	111.8 (3)	C34—C35—C38	108.0 (5)
C4—C3—C2	112.5 (3)	C36—C35—C38	111.5 (6)
C4—C3—H3A	109.1	C34—C35—C37	107.0 (6)
C2—C3—H3A	109.1	C36—C35—C37	109.9 (5)
C4—C3—H3B	109.1	C38—C35—C37	108.9 (4)
C2—C3—H3B	109.1	C35—C36—H36A	109.5
H3A—C3—H3B	107.8	C35—C36—H36B	109.5
C5—C4—C3	112.7 (4)	H36A—C36—H36B	109.5

C5—C4—H4A	109.1	C35—C36—H36C	109.5
C3—C4—H4A	109.1	H36A—C36—H36C	109.5
C5—C4—H4B	109.1	H36B—C36—H36C	109.5
C3—C4—H4B	109.1	C35—C37—H37A	109.5
H4A—C4—H4B	107.8	C35—C37—H37B	109.5
C4—C5—C7	109.1 (4)	H37A—C37—H37B	109.5
C4—C5—C6	110.7 (4)	C35—C37—H37C	109.5
C7—C5—C6	108.8 (4)	H37A—C37—H37C	109.5
C4—C5—C8	108.7 (4)	H37B—C37—H37C	109.5
C7—C5—C8	109.3 (4)	C39—C38—C35	113.5 (4)
C6—C5—C8	110.2 (4)	C39—C38—H38A	108.9
C5—C6—H6A	109.5	C35—C38—H38A	108.9
C5—C6—H6B	109.5	C39—C38—H38B	108.9
H6A—C6—H6B	109.5	C35—C38—H38B	108.9
C5—C6—H6C	109.5	H38A—C38—H38B	107.7
H6A—C6—H6C	109.5	C40—C39—C38	113.5 (4)
H6B—C6—H6C	109.5	C40—C39—C32	111.9 (4)
C5—C7—H7A	109.5	C38—C39—C32	111.0 (4)
C5—C7—H7B	109.5	C40—C39—H39	106.7
H7A—C7—H7B	109.5	C38—C39—H39	106.7
C5—C7—H7C	109.5	C32—C39—H39	106.7
H7A—C7—H7C	109.5	C41—C40—C57	120.7 (4)
H7B—C7—H7C	109.5	C41—C40—C39	119.1 (4)
C5—C8—C9	114.5 (3)	C57—C40—C39	120.2 (4)
C5—C8—H8A	108.6	C40—C41—C42	126.0 (4)
C9—C8—H8A	108.6	C40—C41—H41	117.0
C5—C8—H8B	108.6	C42—C41—H41	117.0
C9—C8—H8B	108.6	C41—C42—C43	113.7 (4)
H8A—C8—H8B	107.6	C41—C42—H42A	108.8
C10—C9—C2	111.4 (3)	C43—C42—H42A	108.8
C10—C9—C8	112.4 (3)	C41—C42—H42B	108.8
C2—C9—C8	110.9 (3)	C43—C42—H42B	108.8
C10—C9—H9	107.3	H42A—C42—H42B	107.7
C2—C9—H9	107.3	C42—C43—C55	109.9 (3)
C8—C9—H9	107.3	C42—C43—C44	114.2 (4)
C11—C10—C9	119.3 (3)	C55—C43—C44	116.9 (4)
C11—C10—C27	119.9 (3)	C42—C43—H43	104.8
C9—C10—C27	120.7 (3)	C55—C43—H43	104.8
C10—C11—C12	126.2 (3)	C44—C43—H43	104.8
C10—C11—H11	116.9	C46—C44—C52	108.6 (3)
C12—C11—H11	116.9	C46—C44—C45	108.1 (4)
C11—C12—C13	114.1 (3)	C52—C44—C45	112.8 (4)
C11—C12—H12A	108.7	C46—C44—C43	107.2 (4)
C13—C12—H12A	108.7	C52—C44—C43	106.5 (3)
C11—C12—H12B	108.7	C45—C44—C43	113.5 (4)
C13—C12—H12B	108.7	C44—C45—H45A	109.5
H12A—C12—H12B	107.6	C44—C45—H45B	109.5
C12—C13—C25	110.4 (3)	H45A—C45—H45B	109.5
C12—C13—C14	113.9 (3)	C44—C45—H45C	109.5

C25—C13—C14	117.4 (3)	H45A—C45—H45C	109.5
C12—C13—H13	104.5	H45B—C45—H45C	109.5
C25—C13—H13	104.5	C47—C46—C44	112.4 (4)
C14—C13—H13	104.5	C47—C46—H46A	109.1
C15—C14—C22	112.8 (3)	C44—C46—H46A	109.1
C15—C14—C16	108.8 (4)	C47—C46—H46B	109.1
C22—C14—C16	106.5 (3)	C44—C46—H46B	109.1
C15—C14—C13	114.2 (3)	H46A—C46—H46B	107.9
C22—C14—C13	107.6 (3)	C48—C47—C46	112.2 (4)
C16—C14—C13	106.5 (3)	C48—C47—H47A	109.2
C14—C15—H15A	109.5	C46—C47—H47A	109.2
C14—C15—H15B	109.5	C48—C47—H47B	109.2
H15A—C15—H15B	109.5	C46—C47—H47B	109.2
C14—C15—H15C	109.5	H47A—C47—H47B	107.9
H15A—C15—H15C	109.5	O6—C48—C47	122.0 (5)
H15B—C15—H15C	109.5	O6—C48—C49	121.3 (5)
C17—C16—C14	112.9 (3)	C47—C48—C49	116.7 (4)
C17—C16—H16A	109.0	C50—C49—C48	108.4 (5)
C14—C16—H16A	109.0	C50—C49—C51	107.9 (5)
C17—C16—H16B	109.0	C48—C49—C51	107.9 (4)
C14—C16—H16B	109.0	C50—C49—C52	110.8 (4)
H16A—C16—H16B	107.8	C48—C49—C52	107.3 (4)
C18—C17—C18'	25.9 (4)	C51—C49—C52	114.3 (4)
C18—C17—C16	114.8 (4)	C49—C50—H50A	109.5
C18'—C17—C16	110.6 (5)	C49—C50—H50B	109.5
C18—C17—H17A	108.6	H50A—C50—H50B	109.5
C18'—C17—H17A	87.1	C49—C50—H50C	109.5
C16—C17—H17A	108.6	H50A—C50—H50C	109.5
C18—C17—H17B	108.6	H50B—C50—H50C	109.5
C18'—C17—H17B	130.7	C49—C51—H51A	109.5
C16—C17—H17B	108.6	C49—C51—H51B	109.5
H17A—C17—H17B	107.5	H51A—C51—H51B	109.5
O3—C18—C17	116.7 (6)	C49—C51—H51C	109.5
O3—C18—C19	117.5 (6)	H51A—C51—H51C	109.5
C17—C18—C19	125.9 (5)	H51B—C51—H51C	109.5
O3'—C18'—C17	115.5 (10)	C53—C52—C44	111.0 (4)
O3'—C18'—C19	122.2 (11)	C53—C52—C49	113.3 (4)
C17—C18'—C19	122.3 (9)	C44—C52—C49	118.8 (4)
C18—C19—C18'	25.2 (3)	C53—C52—H52	104.0
C18—C19—C20	109.4 (4)	C44—C52—H52	104.0
C18'—C19—C20	88.1 (5)	C49—C52—H52	104.0
C18—C19—C21	106.9 (5)	C52—C53—C54	110.1 (4)
C18'—C19—C21	127.8 (5)	C52—C53—H53A	109.6
C20—C19—C21	106.9 (5)	C54—C53—H53A	109.6
C18—C19—C22	110.8 (4)	C52—C53—H53B	109.6
C18'—C19—C22	107.1 (5)	C54—C53—H53B	109.6
C20—C19—C22	108.9 (4)	H53A—C53—H53B	108.2
C21—C19—C22	113.9 (4)	C53—C54—C55	114.6 (4)
C19—C20—H20A	109.5	C53—C54—H54A	108.6

C19—C20—H20B	109.5	C55—C54—H54A	108.6
H20A—C20—H20B	109.5	C53—C54—H54B	108.6
C19—C20—H20C	109.5	C55—C54—H54B	108.6
H20A—C20—H20C	109.5	H54A—C54—H54B	107.6
H20B—C20—H20C	109.5	C56—C55—C54	108.3 (4)
C19—C21—H21A	109.5	C56—C55—C43	110.6 (3)
C19—C21—H21B	109.5	C54—C55—C43	110.5 (3)
H21A—C21—H21B	109.5	C56—C55—C57	110.0 (3)
C19—C21—H21C	109.5	C54—C55—C57	109.9 (3)
H21A—C21—H21C	109.5	C43—C55—C57	107.5 (3)
H21B—C21—H21C	109.5	C55—C56—H56A	109.5
C23—C22—C14	111.7 (3)	C55—C56—H56B	109.5
C23—C22—C19	112.0 (3)	H56A—C56—H56B	109.5
C14—C22—C19	115.8 (3)	C55—C56—H56C	109.5
C23—C22—H22	105.5	H56A—C56—H56C	109.5
C14—C22—H22	105.5	H56B—C56—H56C	109.5
C19—C22—H22	105.5	C40—C57—C59	112.7 (3)
C24—C23—C22	109.6 (3)	C40—C57—C58	106.8 (3)
C24—C23—H23A	109.7	C59—C57—C58	106.8 (3)
C22—C23—H23A	109.7	C40—C57—C55	109.1 (3)
C24—C23—H23B	109.7	C59—C57—C55	109.1 (3)
C22—C23—H23B	109.7	C58—C57—C55	112.3 (3)
H23A—C23—H23B	108.2	C57—C58—H58A	109.5
C23—C24—C25	113.5 (3)	C57—C58—H58B	109.5
C23—C24—H24A	108.9	H58A—C58—H58B	109.5
C25—C24—H24A	108.9	C57—C58—H58C	109.5
C23—C24—H24B	108.9	H58A—C58—H58C	109.5
C25—C24—H24B	108.9	H58B—C58—H58C	109.5
H24A—C24—H24B	107.7	C60—C59—C57	114.6 (4)
C24—C25—C26	108.8 (3)	C60—C59—H59A	108.6
C24—C25—C13	110.4 (3)	C57—C59—H59A	108.6
C26—C25—C13	110.4 (3)	C60—C59—H59B	108.6
C24—C25—C27	109.8 (3)	C57—C59—H59B	108.6
C26—C25—C27	110.2 (3)	H59A—C59—H59B	107.6
C13—C25—C27	107.3 (3)	C32—C60—C59	112.5 (3)
C25—C26—H26A	109.5	C32—C60—H60A	109.1
C25—C26—H26B	109.5	C59—C60—H60A	109.1
H26A—C26—H26B	109.5	C32—C60—H60B	109.1
C25—C26—H26C	109.5	C59—C60—H60B	109.1
H26A—C26—H26C	109.5	H60A—C60—H60B	107.8
H26B—C26—H26C	109.5	C62—C61—H61A	109.5
C10—C27—C29	113.0 (3)	C62—C61—H61B	109.5
C10—C27—C28	106.9 (3)	H61A—C61—H61B	109.5
C29—C27—C28	107.3 (3)	C62—C61—H61C	109.5
C10—C27—C25	108.3 (3)	H61A—C61—H61C	109.5
C29—C27—C25	108.9 (3)	H61B—C61—H61C	109.5
C28—C27—C25	112.6 (3)	C62—C63—C64	107.0 (9)
C27—C28—H28A	109.5	C62—C63—H63A	110.3
C27—C28—H28B	109.5	C64—C63—H63A	110.3

H28A—C28—H28B	109.5	C62—C63—H63B	110.3
C27—C28—H28C	109.5	C64—C63—H63B	110.3
H28A—C28—H28C	109.5	H63A—C63—H63B	108.6
H28B—C28—H28C	109.5	C65—C64—C63	106.6 (9)
C30—C29—C27	114.6 (3)	C65—C64—H64A	110.4
C30—C29—H29A	108.6	C63—C64—H64A	110.4
C27—C29—H29A	108.6	C65—C64—H64B	110.4
C30—C29—H29B	108.6	C63—C64—H64B	110.4
C27—C29—H29B	108.6	H64A—C64—H64B	108.6
H29A—C29—H29B	107.6	C61—C62—C63	110.8 (10)
C29—C30—C2	111.9 (3)	C61—C62—H62A	109.5
C29—C30—H30A	109.2	C63—C62—H62A	109.5
C2—C30—H30A	109.2	C61—C62—H62B	109.5
C29—C30—H30B	109.2	C63—C62—H62B	109.5
C2—C30—H30B	109.2	H62A—C62—H62B	108.1
H30A—C30—H30B	107.9	C66—C65—C64	109.2 (10)
O4—C31—O5	122.0 (4)	C66—C65—H65A	109.8
O4—C31—C32	120.9 (4)	C64—C65—H65A	109.8
O5—C31—C32	117.0 (4)	C66—C65—H65B	109.8
C31—C32—C60	111.9 (4)	C64—C65—H65B	109.8
C31—C32—C39	108.6 (3)	H65A—C65—H65B	108.3
C60—C32—C39	109.4 (3)	C65—C66—H66A	109.5
C31—C32—C33	103.0 (3)	C65—C66—H66B	109.5
C60—C32—C33	111.5 (4)	H66A—C66—H66B	109.5
C39—C32—C33	112.4 (4)	C65—C66—H66C	109.5
C34—C33—C32	112.6 (4)	H66A—C66—H66C	109.5
C34—C33—H33A	109.1	H66B—C66—H66C	109.5
C32—C33—H33A	109.1		
O2—C1—C2—C30	156.5 (4)	C13—C25—C27—C28	57.6 (4)
O1—C1—C2—C30	−27.4 (5)	C10—C27—C29—C30	37.2 (4)
O2—C1—C2—C9	34.1 (5)	C28—C27—C29—C30	−80.3 (4)
O1—C1—C2—C9	−149.9 (3)	C25—C27—C29—C30	157.6 (3)
O2—C1—C2—C3	−85.2 (5)	C27—C29—C30—C2	−53.9 (4)
O1—C1—C2—C3	90.9 (4)	C1—C2—C30—C29	−61.1 (4)
C1—C2—C3—C4	169.1 (4)	C9—C2—C30—C29	61.2 (4)
C30—C2—C3—C4	−71.6 (5)	C3—C2—C30—C29	−175.0 (3)
C9—C2—C3—C4	51.2 (5)	O4—C31—C32—C60	152.6 (5)
C2—C3—C4—C5	−55.4 (5)	O5—C31—C32—C60	−31.2 (6)
C3—C4—C5—C7	175.2 (4)	O4—C31—C32—C39	31.8 (6)
C3—C4—C5—C6	−65.1 (5)	O5—C31—C32—C39	−152.0 (4)
C3—C4—C5—C8	56.1 (5)	O4—C31—C32—C33	−87.5 (6)
C4—C5—C8—C9	−56.1 (5)	O5—C31—C32—C33	88.7 (5)
C7—C5—C8—C9	−175.0 (4)	C31—C32—C33—C34	166.4 (5)
C6—C5—C8—C9	65.3 (5)	C60—C32—C33—C34	−73.4 (6)
C1—C2—C9—C10	70.8 (4)	C39—C32—C33—C34	49.8 (6)
C30—C2—C9—C10	−52.5 (4)	C32—C33—C34—C35	−54.0 (6)
C3—C2—C9—C10	−175.4 (3)	C33—C34—C35—C36	−66.5 (6)
C1—C2—C9—C8	−163.1 (3)	C33—C34—C35—C38	56.2 (6)
C30—C2—C9—C8	73.5 (4)	C33—C34—C35—C37	173.4 (4)

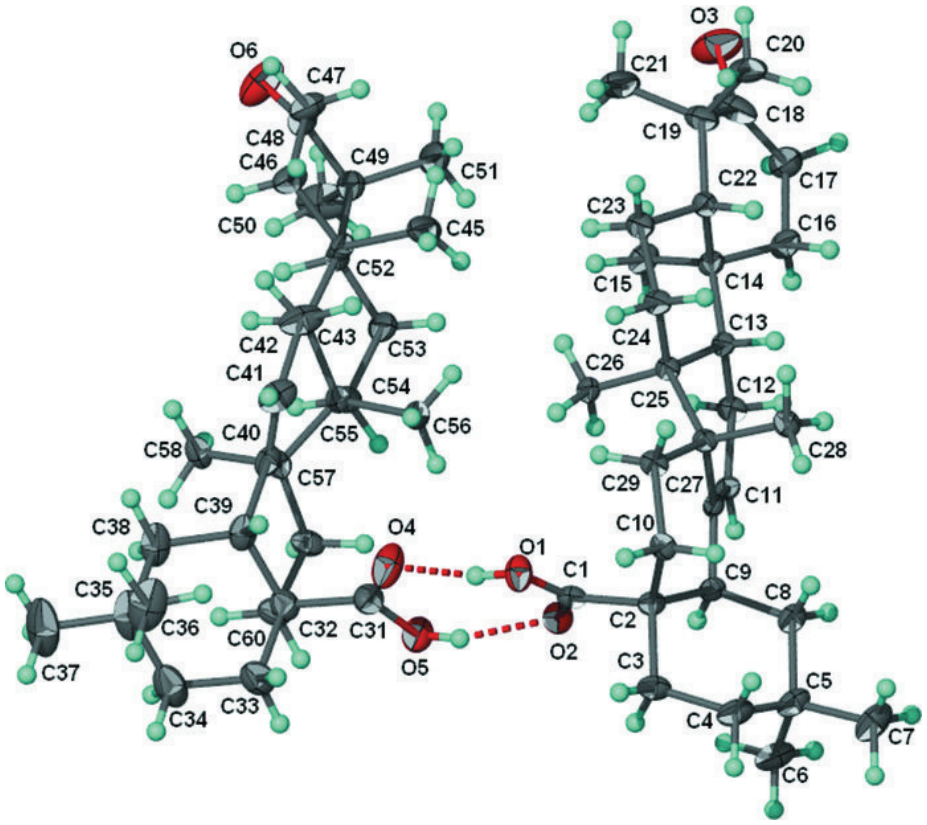
C3—C2—C9—C8	−49.3 (4)	C34—C35—C38—C39	−57.6 (6)
C5—C8—C9—C10	179.2 (4)	C36—C35—C38—C39	65.1 (7)
C5—C8—C9—C2	53.7 (5)	C37—C35—C38—C39	−173.4 (6)
C2—C9—C10—C11	−144.3 (3)	C35—C38—C39—C40	−177.6 (5)
C8—C9—C10—C11	90.4 (4)	C35—C38—C39—C32	55.4 (6)
C2—C9—C10—C27	39.6 (4)	C31—C32—C39—C40	68.9 (4)
C8—C9—C10—C27	−85.6 (4)	C60—C32—C39—C40	−53.4 (4)
C9—C10—C11—C12	−179.0 (3)	C33—C32—C39—C40	−177.8 (3)
C27—C10—C11—C12	−2.9 (6)	C31—C32—C39—C38	−163.2 (4)
C10—C11—C12—C13	2.9 (5)	C60—C32—C39—C38	74.5 (4)
C11—C12—C13—C25	−33.3 (4)	C33—C32—C39—C38	−49.9 (5)
C11—C12—C13—C14	−167.9 (3)	C38—C39—C40—C41	93.5 (5)
C12—C13—C14—C15	54.9 (4)	C32—C39—C40—C41	−139.9 (4)
C25—C13—C14—C15	−76.4 (4)	C38—C39—C40—C57	−84.2 (5)
C12—C13—C14—C22	−179.0 (3)	C32—C39—C40—C57	42.3 (5)
C25—C13—C14—C22	49.6 (4)	C57—C40—C41—C42	−1.8 (7)
C12—C13—C14—C16	−65.2 (4)	C39—C40—C41—C42	−179.5 (4)
C25—C13—C14—C16	163.5 (3)	C40—C41—C42—C43	6.7 (7)
C15—C14—C16—C17	62.0 (5)	C41—C42—C43—C55	−37.9 (5)
C22—C14—C16—C17	−59.8 (5)	C41—C42—C43—C44	−171.6 (4)
C13—C14—C16—C17	−174.4 (4)	C42—C43—C44—C46	−61.7 (5)
C14—C16—C17—C18	24.3 (6)	C55—C43—C44—C46	168.0 (3)
C14—C16—C17—C18'	52.0 (6)	C42—C43—C44—C52	−177.7 (4)
C18'—C17—C18—O3	111.9 (11)	C55—C43—C44—C52	52.0 (4)
C16—C17—C18—O3	−161.8 (5)	C42—C43—C44—C45	57.6 (6)
C18'—C17—C18—C19	−69.0 (11)	C55—C43—C44—C45	−72.7 (5)
C16—C17—C18—C19	17.3 (5)	C52—C44—C46—C47	−52.4 (5)
C18—C17—C18'—O3'	−119.0 (10)	C45—C44—C46—C47	70.2 (5)
C16—C17—C18'—O3'	136.4 (6)	C43—C44—C46—C47	−167.1 (4)
C18—C17—C18'—C19	60.8 (10)	C44—C46—C47—C48	56.1 (5)
C16—C17—C18'—C19	−43.8 (6)	C46—C47—C48—O6	123.7 (6)
O3—C18—C19—C18'	−113.2 (11)	C46—C47—C48—C49	−54.6 (6)
C17—C18—C19—C18'	67.7 (11)	O6—C48—C49—C50	−11.4 (7)
O3—C18—C19—C20	−79.3 (4)	C47—C48—C49—C50	166.9 (5)
C17—C18—C19—C20	101.6 (4)	O6—C48—C49—C51	105.2 (6)
O3—C18—C19—C21	36.0 (4)	C47—C48—C49—C51	−76.5 (6)
C17—C18—C19—C21	−143.1 (4)	O6—C48—C49—C52	−131.1 (5)
O3—C18—C19—C22	160.6 (4)	C47—C48—C49—C52	47.1 (5)
C17—C18—C19—C22	−18.5 (4)	C46—C44—C52—C53	−175.7 (4)
O3'—C18'—C19—C18	118.0 (10)	C45—C44—C52—C53	64.5 (5)
C17—C18'—C19—C18	−61.7 (10)	C43—C44—C52—C53	−60.6 (4)
O3'—C18'—C19—C20	−30.2 (6)	C46—C44—C52—C49	50.3 (5)
C17—C18'—C19—C20	150.0 (5)	C45—C44—C52—C49	−69.5 (5)
O3'—C18'—C19—C21	79.8 (8)	C43—C44—C52—C49	165.4 (3)
C17—C18'—C19—C21	−100.0 (7)	C50—C49—C52—C53	62.5 (5)
O3'—C18'—C19—C22	−139.5 (6)	C48—C49—C52—C53	−179.3 (4)
C17—C18'—C19—C22	40.8 (6)	C51—C49—C52—C53	−59.7 (6)
C15—C14—C22—C23	69.9 (4)	C50—C49—C52—C44	−164.6 (4)
C16—C14—C22—C23	−170.8 (3)	C48—C49—C52—C44	−46.4 (5)

C13—C14—C22—C23	−56.9 (4)	C51—C49—C52—C44	73.2 (6)
C15—C14—C22—C19	−59.8 (4)	C44—C52—C53—C54	64.0 (5)
C16—C14—C22—C19	59.4 (4)	C49—C52—C53—C54	−159.4 (4)
C13—C14—C22—C19	173.3 (3)	C52—C53—C54—C55	−55.3 (5)
C18—C19—C22—C23	−151.5 (3)	C53—C54—C55—C56	−76.9 (5)
C18'—C19—C22—C23	−177.9 (5)	C53—C54—C55—C43	44.4 (5)
C20—C19—C22—C23	88.1 (5)	C53—C54—C55—C57	162.9 (4)
C21—C19—C22—C23	−31.0 (6)	C42—C43—C55—C56	−56.7 (5)
C18—C19—C22—C14	−21.9 (4)	C44—C43—C55—C56	75.7 (5)
C18'—C19—C22—C14	−48.2 (5)	C42—C43—C55—C54	−176.6 (4)
C20—C19—C22—C14	−142.2 (4)	C44—C43—C55—C54	−44.3 (4)
C21—C19—C22—C14	98.7 (5)	C42—C43—C55—C57	63.4 (4)
C14—C22—C23—C24	62.8 (4)	C44—C43—C55—C57	−164.2 (3)
C19—C22—C23—C24	−165.5 (3)	C41—C40—C57—C59	149.0 (4)
C22—C23—C24—C25	−57.7 (4)	C39—C40—C57—C59	−33.3 (5)
C23—C24—C25—C26	−72.9 (4)	C41—C40—C57—C58	−94.0 (5)
C23—C24—C25—C13	48.3 (4)	C39—C40—C57—C58	83.7 (4)
C23—C24—C25—C27	166.4 (3)	C41—C40—C57—C55	27.7 (5)
C12—C13—C25—C24	−178.4 (3)	C39—C40—C57—C55	−154.6 (4)
C14—C13—C25—C24	−45.5 (4)	C56—C55—C57—C40	63.1 (5)
C12—C13—C25—C26	−58.2 (4)	C54—C55—C57—C40	−177.7 (3)
C14—C13—C25—C26	74.7 (4)	C43—C55—C57—C40	−57.4 (4)
C12—C13—C25—C27	61.9 (4)	C56—C55—C57—C59	−60.3 (5)
C14—C13—C25—C27	−165.2 (3)	C54—C55—C57—C59	58.9 (4)
C11—C10—C27—C29	152.6 (3)	C43—C55—C57—C59	179.2 (3)
C9—C10—C27—C29	−31.4 (4)	C56—C55—C57—C58	−178.6 (4)
C11—C10—C27—C28	−89.6 (4)	C54—C55—C57—C58	−59.4 (5)
C9—C10—C27—C28	86.4 (4)	C43—C55—C57—C58	60.9 (4)
C11—C10—C27—C25	31.9 (4)	C40—C57—C59—C60	37.2 (5)
C9—C10—C27—C25	−152.1 (3)	C58—C57—C59—C60	−79.8 (4)
C24—C25—C27—C10	179.6 (3)	C55—C57—C59—C60	158.5 (3)
C26—C25—C27—C10	59.8 (4)	C31—C32—C60—C59	−60.2 (5)
C13—C25—C27—C10	−60.4 (3)	C39—C32—C60—C59	60.2 (5)
C24—C25—C27—C29	56.4 (4)	C33—C32—C60—C59	−174.9 (4)
C26—C25—C27—C29	−63.4 (4)	C57—C59—C60—C32	−52.8 (5)
C13—C25—C27—C29	176.4 (3)	C62—C63—C64—C65	−159 (2)
C24—C25—C27—C28	−62.4 (4)	C64—C63—C62—C61	−73 (2)
C26—C25—C27—C28	177.8 (3)	C63—C64—C65—C66	180 (2)

Hydrogen-bond geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O1—H1···O4	0.84	1.71	2.545 (4)	171
O5—H5···O2	0.84	1.83	2.637 (4)	160
O1w—H1w···O3	0.84	2.20	3.03 (2)	169

Fig. 1


O3
C20
C21
C18
C19
C22
C17
C23
C16
C15
C14
C13
C24
C26
C12
C25
C28
C29
C27
C11
C10
C9
C8
C1
O1
C2
O2
C3
C5
C4
C7
C6
O6
C47
C48
C46
C49
C51
C50
C45
C52
C42
C43
C53
C41
C54
C56
C40
C55
C58
C57
C39
C38
O4
C35
C36
C60
C32
C31
O5
C37
C34
C33

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Pinnatane A from the bark of *Walsura pinnata* Hassk

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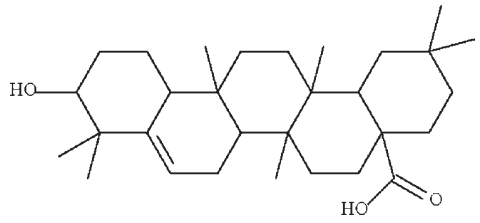
Key indicators: single-crystal X-ray study; T = 100 K; mean σ(C–C) = 0.003 Å; R factor = 0.037; wR factor = 0.097; data-to-parameter ratio = 10.4.

In the molecule of pinnatane A, $C_{30}H_{48}O_3$, isolated from the bark of *Walsura pinnata* Hassk, the four cyclohexane rings adopt chair conformations; the carboxyl and hydroxy substituents occupy axial positions. The cyclohexene ring is envelope-shaped. Adjacent molecules are linked by O—H···O hydrogen bonds into a chain running along the *c* axis.

Related literature

For related structures, see: Awang *et al.* (2009); Jiang *et al.* (1995).



Experimental

Crystal data

$C_{30}H_{48}O_3$
M_r = 456.68
Orthorhombic, $P2_12_12_1$
a = 7.3761 (2) Å
b = 16.3585 (4) Å
c = 20.7032 (5) Å
V = 2498.1 (1) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.08 mm^{-1}
T = 100 K
0.40 × 0.15 × 0.05 mm

Data collection

Bruker SMART APEX diffractometer
Absorption correction: none
17614 measured reflections
3268 independent reflections
2881 reflections with $I > 2\sigma(I)$
R_{int} = 0.045

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.037
$wR(F^2)$ = 0.097
S = 1.00
3268 reflections
313 parameters
2 restraints
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.27 e Å^{-3}
$\Delta\rho_{min}$ = −0.17 e Å^{-3}

Table 1
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1···O3^{i}	0.85 (1)	1.90 (1)	2.731 (2)	167 (3)
O3—H3···O2^{ii}	0.84 (1)	2.36 (2)	3.080 (2)	144 (2)

Symmetry codes: (i) $-x+\frac{1}{2}, -y+2, z-\frac{1}{2}$; (ii) $-x+1, y-\frac{1}{2}, -z+\frac{1}{2}$.

Data collection: *APEX2* (Bruker, 2007); cell refinement: *SAINT* (Bruker, 2007); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *X-SEED* (Barbour, 2001); software used to prepare material for publication: *publCIF* (Westrip, 2009).

We thank the University of Malaya for supporting this study.

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: WN2325).

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supplementary materials

Acta Cryst. (2009). E**65**, o1317 [**doi:10.1107/S1600536809015955**]

Pinnatane A from the bark of *Walsura pinnata* Hassk

K. Mohamad, M. Yusoff, K. Awang, K. Ahmad and S. W. Ng

Comment

Chemicals from *Walsura pinnata* Hassk have not hitherto been reported. We have recently reported the structure of 3-oxoolean-1-en-28-oic acid (Awang *et al.*, 2009), which was obtained from one fraction of the crude extract of the bark of this plant. The last fraction yielded the title compound, which we have named pinnatane A. A related carbon skeleton, assigned from spectroscopic measurements, has been reported (Jiang *et al.*, 1995).

In the molecule of pinnatane A (Fig. 1) the four cyclohexane rings adopt chair comformations, with axial carboxylic acid and hydroxy substituents. The cyclohexene ring is envelope-shaped. Adjacent molecules are linked by O—H···O hydrogen bonds into a chain running along the longest axis of the orthorhombic unit cell.

Experimental

The dried and ground bark of *Walsura pinnata* Hassk (2.3 kg) was extracted with *n*-hexane for 72 h at room temperature. The solvent was evaporated to give a crude extract, which was subjected to column chromatography on silica gel (60 GF254), using *n*-hexane with increasing amounts of ethyl acetate as eluent. Of the twenty-four fractions collected, the twenty-fourth fraction, eluted with ethyl acetate:*n*-hexane (14:86) gave 2 g of the product, which was further purified by column chromatography (*n*-hexane:acetone, 94:6) to give the title compound (5 mg). The formulation was established by satisfactory solution NMR spectroscopy.

Refinement

Carbon-bound H-atoms were placed in calculated positions (C—H 0.95–1.00 Å) and were included in the refinement in the riding model approximation, with U_{iso}(H) set to 1.2–1.5U_{eq}(C). The oxygen-bound H-atoms were located in a difference Fourier map, and were refined with a distance restraint of 0.84±0.01 Å; their displacement parameters were freely refined. In the absence of significant anomalous scattering effects, Friedel pairs were merged.

Figures

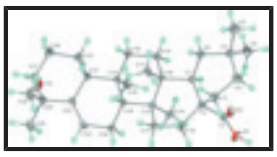


Fig. 1. The molecular structure of the title compound. Displacement ellipsoids are drawn at the 70% probability level. Hydrogen atoms are drawn as spheres of arbitrary radius.

(I)

Crystal data

$C_{30}H_{48}O_3$	F_{000} = 1008
M_r = 456.68	D_x = 1.214 Mg m^{-3}
Orthorhombic, $P2_12_12_1$	Mo $K\alpha$ radiation λ = 0.71073 Å
Hall symbol: P 2ac 2ab	Cell parameters from 3887 reflections
a = 7.3761 (2) Å	θ = 2.3–28.0º
b = 16.3585 (4) Å	μ = 0.08 mm^{-1}
c = 20.7032 (5) Å	T = 100 K
V = 2498.1 (1) Å^3	Chip, colorless
Z = 4	0.40 × 0.15 × 0.05 mm

Data collection

Bruker SMART APEX diffractometer	2881 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	R_{int} = 0.045
Monochromator: graphite	θ_{max} = 27.5º
T = 100 K	θ_{min} = 1.6º
ω scans	h = −9→9
Absorption correction: None	k = −21→21
17614 measured reflections	l = −26→25
3268 independent reflections	

Refinement

Refinement on F^2	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: inferred from neighbouring sites
$R[F^2 > 2\sigma(F^2)]$ = 0.037	H atoms treated by a mixture of independent and constrained refinement
$wR(F^2)$ = 0.097	$w = 1/[\sigma^2(F_o^2) + (0.0608P)^2 + 0.3281P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.00	$(\Delta/\sigma)_{max}$ = 0.001
3268 reflections	$\Delta\rho_{max}$ = 0.27 e Å^{-3}
313 parameters	$\Delta\rho_{min}$ = −0.17 e Å^{-3}
2 restraints	Extinction correction: none
Primary atom site location: structure-invariant direct methods	

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	y	z	U_{iso}*/U_{eq}

O1	0.0192 (2)	1.08616 (9)	−0.04603 (8)	0.0226 (3)
H1	0.022 (5)	1.1379 (6)	−0.0479 (15)	0.054 (9)*
O2	0.2787 (2)	1.10407 (8)	0.00673 (6)	0.0191 (3)
O3	0.5137 (2)	0.74943 (9)	0.43439 (7)	0.0219 (3)
H3	0.590 (3)	0.7286 (16)	0.4595 (11)	0.040 (8)*
C1	0.1662 (3)	1.05839 (11)	−0.01560 (9)	0.0145 (4)
C2	0.1730 (3)	0.96355 (11)	−0.01453 (9)	0.0131 (4)
C3	0.1421 (3)	0.93705 (12)	−0.08573 (9)	0.0168 (4)
H3A	0.1447	0.8766	−0.0884	0.020*
H3B	0.0208	0.9556	−0.0999	0.020*
C4	0.2849 (3)	0.97201 (13)	−0.13099 (10)	0.0209 (4)
H4A	0.2695	1.0321	−0.1333	0.025*
H4B	0.2650	0.9497	−0.1749	0.025*
C5	0.4827 (3)	0.95261 (13)	−0.10968 (9)	0.0197 (4)
C6	0.4780 (3)	0.90007 (12)	−0.04821 (9)	0.0181 (4)
H6A	0.6042	0.8920	−0.0332	0.022*
H6B	0.4290	0.8456	−0.0596	0.022*
C7	0.3656 (3)	0.93451 (11)	0.00866 (9)	0.0129 (4)
H7	0.4306	0.9844	0.0242	0.015*
C8	0.5911 (3)	1.03113 (14)	−0.09846 (11)	0.0267 (5)
H8A	0.5294	1.0649	−0.0661	0.040*
H8B	0.6005	1.0616	−0.1391	0.040*
H8C	0.7129	1.0172	−0.0830	0.040*
C9	0.5782 (4)	0.90337 (16)	−0.16263 (10)	0.0323 (6)
H9A	0.5127	0.8521	−0.1699	0.049*
H9B	0.7028	0.8913	−0.1492	0.049*
H9C	0.5802	0.9352	−0.2027	0.049*
C10	0.3632 (3)	0.87291 (11)	0.06662 (9)	0.0118 (4)
C11	0.3079 (3)	0.78698 (11)	0.04270 (9)	0.0159 (4)
H11A	0.4104	0.7616	0.0203	0.024*
H11B	0.2051	0.7918	0.0129	0.024*
H11C	0.2729	0.7531	0.0797	0.024*
C12	0.2315 (3)	0.90314 (11)	0.12067 (8)	0.0116 (4)
C13	0.0361 (3)	0.89617 (12)	0.09446 (9)	0.0148 (4)
H13A	−0.0464	0.9250	0.1245	0.018*
H13B	0.0009	0.8378	0.0943	0.018*
C14	0.0073 (3)	0.93116 (12)	0.02569 (9)	0.0172 (4)
H14A	−0.0805	0.9767	0.0294	0.021*
H14B	−0.0520	0.8880	−0.0003	0.021*
C15	0.2605 (3)	0.99440 (11)	0.13609 (9)	0.0151 (4)
H15A	0.2138	1.0063	0.1794	0.023*
H15B	0.1958	1.0278	0.1043	0.023*
H15C	0.3902	1.0071	0.1344	0.023*
C16	0.2508 (3)	0.84795 (11)	0.18263 (9)	0.0116 (4)
H16	0.2122	0.7920	0.1688	0.014*
C17	0.4463 (3)	0.83731 (11)	0.20946 (9)	0.0126 (4)
C18	0.5718 (3)	0.81296 (12)	0.15331 (9)	0.0147 (4)
H18A	0.6988	0.8135	0.1688	0.018*
H18B	0.5427	0.7563	0.1400	0.018*

C19	0.5566 (3)	0.86876 (12)	0.09454 (9)	0.0147 (4)
H19A	0.6402	0.8489	0.0606	0.018*
H19B	0.5955	0.9245	0.1069	0.018*
C20	0.5197 (3)	0.91447 (11)	0.24330 (10)	0.0168 (4)
H20A	0.4518	0.9239	0.2833	0.025*
H20B	0.5057	0.9617	0.2146	0.025*
H20C	0.6484	0.9068	0.2535	0.025*
C21	0.4437 (3)	0.76507 (11)	0.25864 (9)	0.0136 (4)
H21	0.4314	0.7139	0.2325	0.016*
C22	0.2839 (3)	0.76640 (11)	0.30481 (9)	0.0139 (4)
C23	0.1435 (3)	0.81574 (12)	0.29524 (9)	0.0150 (4)
H23	0.0502	0.8161	0.3269	0.018*
C24	0.1219 (3)	0.87123 (12)	0.23765 (9)	0.0151 (4)
H24A	0.1461	0.9283	0.2509	0.018*
H24B	−0.0047	0.8682	0.2220	0.018*
C25	0.6225 (3)	0.75728 (12)	0.29622 (10)	0.0182 (4)
H25A	0.7252	0.7543	0.2656	0.022*
H25B	0.6397	0.8060	0.3239	0.022*
C26	0.6192 (3)	0.68067 (12)	0.33802 (10)	0.0191 (4)
H26A	0.5993	0.6321	0.3104	0.023*
H26B	0.7377	0.6742	0.3599	0.023*
C27	0.4700 (3)	0.68582 (11)	0.38820 (9)	0.0168 (4)
H27	0.4658	0.6325	0.4118	0.020*
C28	0.2819 (3)	0.70122 (11)	0.35883 (9)	0.0156 (4)
C29	0.1487 (3)	0.72131 (13)	0.41332 (9)	0.0199 (4)
H29A	0.1724	0.7766	0.4294	0.030*
H29B	0.1641	0.6820	0.4486	0.030*
H29C	0.0243	0.7182	0.3969	0.030*
C30	0.2190 (3)	0.62008 (12)	0.32797 (10)	0.0215 (4)
H30A	0.3000	0.6059	0.2922	0.032*
H30B	0.0950	0.6263	0.3117	0.032*
H30C	0.2221	0.5766	0.3605	0.032*

Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0198 (8)	0.0148 (7)	0.0332 (8)	0.0020 (6)	−0.0087 (7)	0.0031 (6)
O2	0.0224 (8)	0.0151 (6)	0.0198 (7)	−0.0031 (6)	−0.0050 (6)	0.0014 (5)
O3	0.0293 (9)	0.0174 (7)	0.0190 (7)	0.0024 (7)	−0.0108 (7)	−0.0029 (6)
C1	0.0152 (10)	0.0161 (9)	0.0121 (8)	0.0021 (8)	0.0016 (8)	0.0006 (7)
C2	0.0123 (9)	0.0140 (8)	0.0130 (9)	0.0006 (7)	−0.0012 (8)	0.0005 (7)
C3	0.0186 (10)	0.0184 (9)	0.0134 (9)	0.0001 (8)	−0.0033 (8)	−0.0014 (7)
C4	0.0216 (11)	0.0284 (11)	0.0126 (9)	0.0043 (9)	−0.0013 (9)	0.0004 (8)
C5	0.0208 (11)	0.0254 (10)	0.0128 (9)	0.0072 (9)	0.0044 (9)	0.0035 (8)
C6	0.0184 (10)	0.0223 (10)	0.0136 (9)	0.0065 (9)	0.0025 (8)	0.0030 (8)
C7	0.0119 (9)	0.0146 (9)	0.0121 (8)	0.0012 (7)	0.0002 (7)	0.0005 (7)
C8	0.0181 (11)	0.0348 (12)	0.0273 (11)	−0.0002 (10)	0.0018 (9)	0.0123 (10)
C9	0.0379 (15)	0.0426 (14)	0.0165 (10)	0.0175 (12)	0.0082 (10)	0.0050 (10)

C10	0.0106 (9)	0.0128 (8)	0.0119 (8)	0.0012 (7)	0.0012 (7)	0.0009 (7)
C11	0.0175 (10)	0.0141 (9)	0.0160 (9)	0.0010 (8)	0.0010 (8)	−0.0008 (7)
C12	0.0111 (9)	0.0126 (8)	0.0112 (8)	0.0006 (7)	−0.0005 (7)	0.0001 (6)
C13	0.0120 (9)	0.0170 (9)	0.0155 (9)	0.0012 (8)	0.0008 (8)	0.0034 (7)
C14	0.0131 (10)	0.0195 (9)	0.0190 (9)	−0.0007 (8)	−0.0017 (8)	0.0045 (8)
C15	0.0166 (10)	0.0142 (9)	0.0144 (8)	0.0014 (8)	−0.0006 (8)	0.0004 (7)
C16	0.0114 (9)	0.0105 (8)	0.0129 (8)	−0.0003 (7)	0.0009 (7)	0.0016 (6)
C17	0.0115 (9)	0.0135 (9)	0.0128 (9)	0.0009 (7)	0.0001 (7)	0.0018 (7)
C18	0.0116 (10)	0.0172 (9)	0.0151 (9)	0.0023 (8)	0.0009 (7)	0.0031 (7)
C19	0.0128 (10)	0.0167 (9)	0.0146 (9)	0.0005 (8)	0.0008 (8)	0.0014 (7)
C20	0.0169 (10)	0.0160 (9)	0.0175 (9)	−0.0023 (8)	−0.0021 (8)	0.0013 (7)
C21	0.0147 (10)	0.0128 (9)	0.0134 (9)	0.0008 (7)	−0.0001 (7)	0.0007 (7)
C22	0.0168 (10)	0.0136 (8)	0.0114 (8)	−0.0035 (7)	−0.0014 (8)	−0.0003 (7)
C23	0.0141 (10)	0.0187 (9)	0.0122 (8)	−0.0008 (8)	0.0021 (8)	−0.0002 (7)
C24	0.0121 (10)	0.0184 (9)	0.0150 (9)	0.0025 (8)	0.0015 (8)	0.0025 (7)
C25	0.0152 (10)	0.0188 (10)	0.0205 (10)	0.0013 (8)	−0.0003 (8)	0.0053 (8)
C26	0.0206 (11)	0.0184 (10)	0.0183 (9)	0.0033 (9)	−0.0030 (9)	0.0032 (8)
C27	0.0245 (11)	0.0126 (9)	0.0133 (9)	0.0000 (8)	−0.0028 (8)	0.0001 (7)
C28	0.0198 (10)	0.0137 (9)	0.0131 (9)	−0.0019 (8)	−0.0007 (8)	−0.0002 (7)
C29	0.0225 (11)	0.0236 (10)	0.0136 (9)	−0.0015 (9)	0.0011 (8)	0.0038 (8)
C30	0.0281 (12)	0.0192 (10)	0.0172 (9)	−0.0063 (9)	−0.0039 (9)	0.0020 (8)

Geometric parameters (Å, °)

O1—C1	1.334 (2)	C15—H15A	0.9800
O1—H1	0.85 (1)	C15—H15B	0.9800
O2—C1	1.208 (2)	C15—H15C	0.9800
O3—C27	1.450 (2)	C16—C24	1.532 (3)
O3—H3	0.84 (1)	C16—C17	1.555 (3)
C1—C2	1.552 (3)	C16—H16	1.0000
C2—C3	1.553 (3)	C17—C18	1.538 (3)
C2—C14	1.571 (3)	C17—C20	1.542 (3)
C2—C7	1.573 (3)	C17—C21	1.560 (2)
C3—C4	1.521 (3)	C18—C19	1.525 (2)
C3—H3A	0.9900	C18—H18A	0.9900
C3—H3B	0.9900	C18—H18B	0.9900
C4—C5	1.557 (3)	C19—H19A	0.9900
C4—H4A	0.9900	C19—H19B	0.9900
C4—H4B	0.9900	C20—H20A	0.9800
C5—C9	1.532 (3)	C20—H20B	0.9800
C5—C8	1.531 (3)	C20—H20C	0.9800
C5—C6	1.536 (3)	C21—C22	1.518 (3)
C6—C7	1.546 (3)	C21—C25	1.536 (3)
C6—H6A	0.9900	C21—H21	1.0000
C6—H6B	0.9900	C22—C23	1.328 (3)
C7—C10	1.567 (2)	C22—C28	1.545 (2)
C7—H7	1.0000	C23—C24	1.507 (2)
C8—H8A	0.9800	C23—H23	0.9500
C8—H8B	0.9800	C24—H24A	0.9900

C8—H8C	0.9800	C24—H24B	0.9900
C9—H9A	0.9800	C25—C26	1.523 (3)
C9—H9B	0.9800	C25—H25A	0.9900
C9—H9C	0.9800	C25—H25B	0.9900
C10—C19	1.541 (3)	C26—C27	1.516 (3)
C10—C11	1.545 (3)	C26—H26A	0.9900
C10—C12	1.562 (3)	C26—H26B	0.9900
C11—H11A	0.9800	C27—C28	1.535 (3)
C11—H11B	0.9800	C27—H27	1.0000
C11—H11C	0.9800	C28—C29	1.532 (3)
C12—C15	1.542 (3)	C28—C30	1.544 (3)
C12—C13	1.544 (3)	C29—H29A	0.9800
C12—C16	1.575 (2)	C29—H29B	0.9800
C13—C14	1.549 (3)	C29—H29C	0.9800
C13—H13A	0.9900	C30—H30A	0.9800
C13—H13B	0.9900	C30—H30B	0.9800
C14—H14A	0.9900	C30—H30C	0.9800
C14—H14B	0.9900		
C1—O1—H1	110 (2)	H15A—C15—H15C	109.5
C27—O3—H3	105.5 (19)	H15B—C15—H15C	109.5
O2—C1—O1	121.87 (17)	C24—C16—C17	109.75 (15)
O2—C1—C2	126.19 (18)	C24—C16—C12	114.01 (15)
O1—C1—C2	111.93 (17)	C17—C16—C12	116.04 (15)
C1—C2—C3	105.10 (15)	C24—C16—H16	105.3
C1—C2—C14	108.63 (16)	C17—C16—H16	105.3
C3—C2—C14	107.14 (16)	C12—C16—H16	105.3
C1—C2—C7	109.60 (16)	C18—C17—C20	110.12 (16)
C3—C2—C7	109.74 (16)	C18—C17—C16	108.48 (15)
C14—C2—C7	116.04 (15)	C20—C17—C16	113.35 (16)
C4—C3—C2	112.22 (16)	C18—C17—C21	107.73 (15)
C4—C3—H3A	109.2	C20—C17—C21	109.15 (15)
C2—C3—H3A	109.2	C16—C17—C21	107.84 (15)
C4—C3—H3B	109.2	C19—C18—C17	113.81 (15)
C2—C3—H3B	109.2	C19—C18—H18A	108.8
H3A—C3—H3B	107.9	C17—C18—H18A	108.8
C3—C4—C5	113.42 (16)	C19—C18—H18B	108.8
C3—C4—H4A	108.9	C17—C18—H18B	108.8
C5—C4—H4A	108.9	H18A—C18—H18B	107.7
C3—C4—H4B	108.9	C18—C19—C10	113.18 (16)
C5—C4—H4B	108.9	C18—C19—H19A	108.9
H4A—C4—H4B	107.7	C10—C19—H19A	108.9
C9—C5—C8	108.03 (19)	C18—C19—H19B	108.9
C9—C5—C6	108.00 (16)	C10—C19—H19B	108.9
C8—C5—C6	110.83 (17)	H19A—C19—H19B	107.8
C9—C5—C4	109.59 (18)	C17—C20—H20A	109.5
C8—C5—C4	111.19 (17)	C17—C20—H20B	109.5
C6—C5—C4	109.13 (17)	H20A—C20—H20B	109.5
C5—C6—C7	116.03 (16)	C17—C20—H20C	109.5
C5—C6—H6A	108.3	H20A—C20—H20C	109.5

C7—C6—H6A	108.3	H20B—C20—H20C	109.5
C5—C6—H6B	108.3	C22—C21—C25	110.42 (15)
C7—C6—H6B	108.3	C22—C21—C17	114.20 (15)
H6A—C6—H6B	107.4	C25—C21—C17	112.50 (16)
C6—C7—C10	110.77 (15)	C22—C21—H21	106.4
C6—C7—C2	111.23 (15)	C25—C21—H21	106.4
C10—C7—C2	114.71 (15)	C17—C21—H21	106.4
C6—C7—H7	106.5	C23—C22—C21	121.36 (16)
C10—C7—H7	106.5	C23—C22—C28	121.35 (18)
C2—C7—H7	106.5	C21—C22—C28	116.93 (16)
C5—C8—H8A	109.5	C22—C23—C24	124.52 (18)
C5—C8—H8B	109.5	C22—C23—H23	117.7
H8A—C8—H8B	109.5	C24—C23—H23	117.7
C5—C8—H8C	109.5	C23—C24—C16	111.90 (16)
H8A—C8—H8C	109.5	C23—C24—H24A	109.2
H8B—C8—H8C	109.5	C16—C24—H24A	109.2
C5—C9—H9A	109.5	C23—C24—H24B	109.2
C5—C9—H9B	109.5	C16—C24—H24B	109.2
H9A—C9—H9B	109.5	H24A—C24—H24B	107.9
C5—C9—H9C	109.5	C26—C25—C21	110.02 (17)
H9A—C9—H9C	109.5	C26—C25—H25A	109.7
H9B—C9—H9C	109.5	C21—C25—H25A	109.7
C19—C10—C11	108.97 (16)	C26—C25—H25B	109.7
C19—C10—C12	108.72 (14)	C21—C25—H25B	109.7
C11—C10—C12	110.67 (16)	H25A—C25—H25B	108.2
C19—C10—C7	107.78 (15)	C27—C26—C25	110.80 (17)
C11—C10—C7	110.03 (15)	C27—C26—H26A	109.5
C12—C10—C7	110.60 (14)	C25—C26—H26A	109.5
C10—C11—H11A	109.5	C27—C26—H26B	109.5
C10—C11—H11B	109.5	C25—C26—H26B	109.5
H11A—C11—H11B	109.5	H26A—C26—H26B	108.1
C10—C11—H11C	109.5	O3—C27—C26	109.29 (17)
H11A—C11—H11C	109.5	O3—C27—C28	110.14 (16)
H11B—C11—H11C	109.5	C26—C27—C28	113.18 (16)
C15—C12—C13	105.88 (16)	O3—C27—H27	108.0
C15—C12—C10	111.64 (15)	C26—C27—H27	108.0
C13—C12—C10	107.79 (14)	C28—C27—H27	108.0
C15—C12—C16	111.95 (14)	C29—C28—C27	108.85 (15)
C13—C12—C16	109.17 (15)	C29—C28—C30	107.23 (17)
C10—C12—C16	110.22 (14)	C27—C28—C30	107.11 (16)
C12—C13—C14	115.06 (16)	C29—C28—C22	113.01 (16)
C12—C13—H13A	108.5	C27—C28—C22	113.06 (16)
C14—C13—H13A	108.5	C30—C28—C22	107.25 (15)
C12—C13—H13B	108.5	C28—C29—H29A	109.5
C14—C13—H13B	108.5	C28—C29—H29B	109.5
H13A—C13—H13B	107.5	H29A—C29—H29B	109.5
C13—C14—C2	120.34 (17)	C28—C29—H29C	109.5
C13—C14—H14A	107.2	H29A—C29—H29C	109.5
C2—C14—H14A	107.2	H29B—C29—H29C	109.5

C13—C14—H14B	107.2	C28—C30—H30A	109.5
C2—C14—H14B	107.2	C28—C30—H30B	109.5
H14A—C14—H14B	106.9	H30A—C30—H30B	109.5
C12—C15—H15A	109.5	C28—C30—H30C	109.5
C12—C15—H15B	109.5	H30A—C30—H30C	109.5
H15A—C15—H15B	109.5	H30B—C30—H30C	109.5
C12—C15—H15C	109.5		
O2—C1—C2—C3	−128.3 (2)	C15—C12—C16—C17	−72.0 (2)
O1—C1—C2—C3	50.5 (2)	C13—C12—C16—C17	171.15 (15)
O2—C1—C2—C14	117.3 (2)	C10—C12—C16—C17	52.9 (2)
O1—C1—C2—C14	−63.9 (2)	C24—C16—C17—C18	179.31 (15)
O2—C1—C2—C7	−10.4 (3)	C12—C16—C17—C18	−49.6 (2)
O1—C1—C2—C7	168.37 (15)	C24—C16—C17—C20	−58.05 (19)
C1—C2—C3—C4	59.0 (2)	C12—C16—C17—C20	72.99 (19)
C14—C2—C3—C4	174.41 (16)	C24—C16—C17—C21	62.89 (19)
C7—C2—C3—C4	−58.8 (2)	C12—C16—C17—C21	−166.06 (14)
C2—C3—C4—C5	54.0 (2)	C20—C17—C18—C19	−73.7 (2)
C3—C4—C5—C9	119.69 (19)	C16—C17—C18—C19	50.8 (2)
C3—C4—C5—C8	−120.95 (19)	C21—C17—C18—C19	167.33 (16)
C3—C4—C5—C6	1.6 (2)	C17—C18—C19—C10	−58.2 (2)
C9—C5—C6—C7	−172.55 (19)	C11—C10—C19—C18	−62.8 (2)
C8—C5—C6—C7	69.3 (2)	C12—C10—C19—C18	57.9 (2)
C4—C5—C6—C7	−53.5 (2)	C7—C10—C19—C18	177.82 (15)
C5—C6—C7—C10	176.81 (17)	C18—C17—C21—C22	−161.11 (16)
C5—C6—C7—C2	48.0 (2)	C20—C17—C21—C22	79.3 (2)
C1—C2—C7—C6	−106.48 (18)	C16—C17—C21—C22	−44.2 (2)
C3—C2—C7—C6	8.5 (2)	C18—C17—C21—C25	72.02 (19)
C14—C2—C7—C6	130.06 (17)	C20—C17—C21—C25	−47.5 (2)
C1—C2—C7—C10	126.83 (16)	C16—C17—C21—C25	−171.08 (15)
C3—C2—C7—C10	−118.23 (16)	C25—C21—C22—C23	140.08 (18)
C14—C2—C7—C10	3.4 (2)	C17—C21—C22—C23	12.1 (2)
C6—C7—C10—C19	67.88 (19)	C25—C21—C22—C28	−46.6 (2)
C2—C7—C10—C19	−165.19 (16)	C17—C21—C22—C28	−174.56 (16)
C6—C7—C10—C11	−50.8 (2)	C21—C22—C23—C24	3.2 (3)
C2—C7—C10—C11	76.1 (2)	C28—C22—C23—C24	−169.77 (18)
C6—C7—C10—C12	−173.40 (16)	C22—C23—C24—C16	15.8 (3)
C2—C7—C10—C12	−46.5 (2)	C17—C16—C24—C23	−48.9 (2)
C19—C10—C12—C15	71.33 (18)	C12—C16—C24—C23	178.98 (15)
C11—C10—C12—C15	−169.02 (15)	C22—C21—C25—C26	57.1 (2)
C7—C10—C12—C15	−46.8 (2)	C17—C21—C25—C26	−173.99 (16)
C19—C10—C12—C13	−172.80 (15)	C21—C25—C26—C27	−62.7 (2)
C11—C10—C12—C13	−53.15 (19)	C25—C26—C27—O3	−67.6 (2)
C7—C10—C12—C13	69.05 (18)	C25—C26—C27—C28	55.6 (2)
C19—C10—C12—C16	−53.74 (19)	O3—C27—C28—C29	−46.8 (2)
C11—C10—C12—C16	65.91 (19)	C26—C27—C28—C29	−169.46 (16)
C7—C10—C12—C16	−171.90 (15)	O3—C27—C28—C30	−162.41 (16)
C15—C12—C13—C14	72.33 (19)	C26—C27—C28—C30	74.91 (19)
C10—C12—C13—C14	−47.3 (2)	O3—C27—C28—C22	79.67 (19)
C16—C12—C13—C14	−166.99 (15)	C26—C27—C28—C22	−43.0 (2)

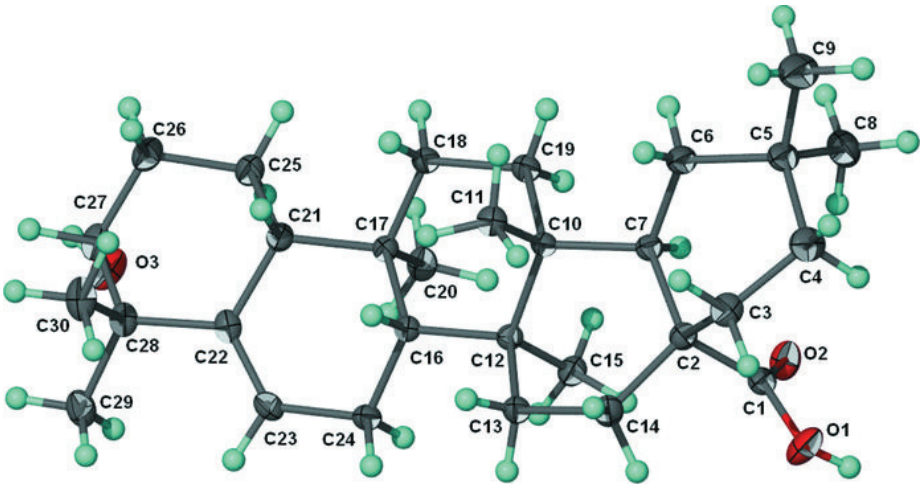
C12—C13—C14—C2	5.1 (3)	C23—C22—C28—C29	−22.9 (3)
C1—C2—C14—C13	−106.01 (19)	C21—C22—C28—C29	163.82 (16)
C3—C2—C14—C13	140.93 (18)	C23—C22—C28—C27	−147.09 (18)
C7—C2—C14—C13	18.0 (2)	C21—C22—C28—C27	39.6 (2)
C15—C12—C16—C24	57.0 (2)	C23—C22—C28—C30	95.1 (2)
C13—C12—C16—C24	−59.8 (2)	C21—C22—C28—C30	−78.2 (2)
C10—C12—C16—C24	−178.05 (15)		

Hydrogen-bond geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O1—H1···O3^{i}	0.85 (1)	1.90 (1)	2.731 (2)	167 (3)
O3—H3···O2^{ii}	0.84 (1)	2.36 (2)	3.080 (2)	144 (2)

Symmetry codes: (i) −*x*+1/2, −*y*+2, *z*−1/2; (ii) −*x*+1, *y*−1/2, −*z*+1/2.

Fig. 1


C9
C26
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C4
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O2
C29
C1
C23
C24
C13
C14
O1